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ELECTROCARDIOGRAPH STUDIES IN NORMAL DAIRY CATTLE[1]

By B. V. ALFREDSON, *assistant professor of physiology and pharmacology*, and J. F. SYKES, *research assistant in physiology, Michigan Agricultural Experiment Station*[2]

INTRODUCTION

Since the introduction of the electrocardiograph by Einthoven nearly 30 years ago, the study of heart conditions in the human subject has made tremendous strides. With this instrument it is possible to record the electrical effects which accompany each beat of the heart. In the human subject a regular series of waves is recorded in succession as the several regions of the heart become active. These waves are arbitrarily designated as P, Q, R, S, and T, the order in which they occur during each heart beat. Their form is shown in figure 1.

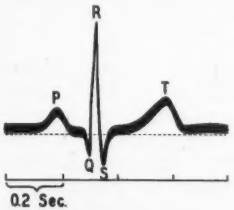

FIGURE 1.—Form of the waves in a human heart beat, in the order in which they occur, P, Q, R, S, and T. Waves above the dotted isoelectric line in figure 1 are designated as positive deflections while those that go below are negative. The P wave is formed during auricular activity and Q, R, S, and T arise during ventricular activity. While there are some variations in the form of these waves among normal individuals, certain variations from the normal are very significant and may be used to detect abnormal conditions of the heart.

By means of a timing device the electrocardiograph also enables the clinician to determine the length of time necessary for the completion of the various phases of the cardiac cycle. The PR interval represents the time occupied by auricular activity, and the QT interval that occupied by ventricular activity. Variations in the length of these intervals indicate cardiac abnormalities. It is possible to determine from the record the relative height or potential

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of the various waves. Variations in potential may under certain circumstances have significant diagnostic value.

The present paper reports the results of an attempt to establish normal electrocardiographic values for cattle as a basis for experimental and clinical investigation in this species. Relatively few reports on this subject have appeared in the literature, and most of these have come from Europe (*12*, *17*, *18*, *22*, *23*, *25*).[3] In only two instances known to the writers has similar work been reported in the United States (*3*, *20*). With the establishment of normal electrocardiographic values it seems probable that the electrocardiograph may become a valuable tool in experimental studies on bovines and possibly in time it may have a limited use in clinical veterinary medicine.

EXPERIMENTAL PROCEDURE

Ninety-seven animals, composing the major part of the Michigan State College dairy herd, were selected for study. The breed representation was as follows: Jersey, 23; Guernsey, 22; Ayrshire, 13; Brown Swiss, 19; Holstein, 20. These animals were representative of their various breeds and were kept on the fairly high plane of nutrition necessary for optimum production under modern conditions of herd management. The age distribution ranged from 5 months to 12 years, inclusive. The animals were in various stages of gestation and lactation.

Three serial electrocardiograms, approximately 1 month apart, were recorded for each individual. The animals were accustomed to being handled and were given a reasonable opportunity to adjust themselves to the new surroundings of the laboratory before the tracings were taken. Since changes in heart rate may affect the QT and the PR intervals, no recordings were attempted when the animals showed any evidence of excitement. All records were taken in the afternoon.

The apparatus employed was the Hindle No. 2 model of the Einthoven electrocardiograph. The three standard leads only were employed; namely, lead I, right front leg to left front leg; lead II, right front leg to left rear leg; lead III, left front leg to left rear leg. These leads were chosen despite the fact that their relative positions in respect to the plane of the long axis of the bovine heart is considerably different from the position of the same leads in respect to the long axis of the heart in the human subject. Such leads would not be expected to produce electrocardiograms closely resembling those of human subjects,[4] and for this reason, they have been discarded by others. Nörr (*17*, *18*) and Kahn (*12*) employed a single lead and Yacoël and Spitz (*25*), attempted to place the electrodes in such a manner as to bring the leads into a relationship with the long axis

[3] Italic numbers in parentheses refer to Literature Cited, p. 85.

[4] In the human subject the electrical effects produced by cardiac activity are picked up by electrodes attached to the arms and the left leg. The electrodes are connected to the galvanometer, and a control box enables the operator to pick up the electrical effects from any two electrodes and direct them through the galvanometer. Each such circuit when completed is known as a lead. With three electrodes placed as noted, three leads are possible. The form of the various waves of the electrocardiogram is partly due to the position of the heart in relation to the point of attachment of the electrodes or the leads. Because of anatomical differences in the human subject and the bovine it is apparent that the position of the heart in relation to the leads would be considerably different and the form of the various waves would also be somewhat different.

of the heart similar to that obtained in the human subject. The single-lead electrocardiograms did not show as marked an individual variation as electrocardiograms taken with the standard leads, but still they did not closely resemble those of human subjects, and, while the method of Yacoël and Spitz met this condition to a certain extent, they attained this result only by moving the electrodes to new positions for each lead. The use of the three standard leads is therefore justified partly because they afford more extensive information than can be obtained from the single leads employed by Nörr (*17*, *18*) and Kahn (*12*) and partly because they help considerably to simplify the problem of electrode application.

The Cambridge german silver plate electrodes were used exclusively. The area of application was clipped and electrode paste was applied and rubbed vigorously. At no time during the recording of an electrocardiogram were the subject's feet allowed to make a wet contact with the floor. In this study the nomenclature introduced by Einthoven was employed and the letters P, Q, R, S, and T were assigned to the various electrocardiographic deflections according to the rules in common use in human electrocardiography. A second upright QRS deflection encountered in certain cases was designated R′.

Since the heart rate has been shown to influence especially the QT, and to a lesser extent the PR, interval in the human subject, it was necessary to determine rates so that the effect of this factor on the intervals could be determined. The heart rate in each lead was found by taking an average of five or six RR intervals. The values obtained in this way in each lead of the three monthly tracings (nine values in all) were averaged and the value so derived was expressed as the heart rate of that particular individual.

The intervals PR, QRS, and QT were measured under magnification by a reading glass. The accuracy of this method is approximately 0.01 second for all intervals. Readable complexes were always selected for measurement of intervals. Contrary to the usual custom in investigations with human subjects, the arbitrary selection of any given lead for determination of interval length is practically impossible because of the extreme range of the cardiac electrical axis in the bovine. For the sake of uniformity and since the longest interval in any lead of the first monthly tracing was rarely exceeded in the two subsequent records, the greatest interval in any lead of the first tracing was arbitrarily selected for tabulation unless otherwise indicated.

The systolic index or systole-cycle ratio was calculated for each electrocardiogram according to the formula devised by Bazett (*4*), which is as follows:

$$K=\frac{QT^{1}}{\sqrt{RR}}$$

The value of K is commonly considered as practically constant in normal human electrocardiograms (*7*). Any variation in K represents an alteration of the relationship of QT to the entire cycle, whereas

[1] QT is the time interval from the beginning of QRS to the end of T, and RR is the interval in seconds occupied by a complete cardiac cycle as exemplified by the time interval between two successive R waves.

measurement of the QT interval might reveal no actual change.[6] Such changes in the human electrocardiogram have some clinical significance.

The cardiac electrical axis,[7] which is based upon the variations in the potential of the QRS deflections between the leads, was determined by approximate methods in the present study. By using a series of triangular diagrams based upon the recommendation of Wilson (*24*) and representing axis gradations in steps of 30°, the approximate electrical axis of each electrocardiogram in the series was determined. The only criterion for the evaluation of QRS for this purpose was an estimation of the approximate net area of its deflection as nearly as this could be determined by observation. No attempt was made to compare the potential in the three leads at the same relative time instant by using graphic methods of the type employed, for example, by Fahr (*9*), nor was any attempt made to determine the manifest potential according to the method of Einthoven (*8*). These techniques are probably more accurate than the one employed in the present study, but they cannot be used with precision until more is known concerning the electrical effects which accompany the heart beat of the bovine subject.

RESULTS AND DISCUSSION

THE HEART RATE

Table 1 gives a summary of the data derived from the individual records; for purposes of comparison a summary of data compiled by Fuller (*11*) is included. Heart rates varying from 48 to 98 beats per minute were observed. The average rate was 71.6 beats per minute for the entire series. The mean for the breeds ranged from 65.9 for the Jerseys to 76.4 for the Ayrshires. These values are somewhat higher than those reported by Fuller. The difference appears to be due to the fact that 25 individuals in this group of cattle ranged in age from 5 to 18 months. The heart rates of these younger cattle were found to avergae 82.5 beats per minute while the average for the animals over 1½ years of age was 67.8 (table 2). The latter figure agrees very closely with the average given by Fuller.

In spite of the fact that many factors may affect heart rate, the values given in table 2 may be considered to be within normal limits for cattle in the two age groups. The fact that these animsls were led from one barn to another, a distance of 300 yards, before the record was taken would not seriously affect the rate. It has been demonstrated by Lewis and Cotton (*15*) that when the human heart rate is increased by exercise it returns to normal in from 3 to 5 minutes. Usually from 10 to 15 minutes elapsed between the arrival of the animal at the laboratory and the actual taking of tracings. Wide fluctua-

[6] Since the QT interval varies with the rate of the heart it is desirable to have some means of comparing QT at various rate levels. For instance, if the QT interval in a given subject with a heart rate of 70 were found to be 0.40 second and a subsequent record of the same subject gave a QT interval of 0.40 second when the heart rate was found to be 50, it is apparent that the QT interval in the first instance is proportionately larger in relation to the whole cardiac cycle than it is in the second although the actual measurements by themselves would not indicate such to be the case, and therefore, these two values for QT are not comparable. Bazett's formula is used in human studies to correct for variations in rate which may affect the QT interval, and the values obtained by use of the formula make it possible to compare QT at various cardiac frequencies.

[7] The electromotive forces developed in the heart at any one instant have a certain definite direction of flow which is known as the electrical axis of the heart. The direction at any instant depends on activity in one part and quiescence in other parts; i. e., from electrically negative to electrically positive areas. Variations in the electrical axis represent changes in the direction of flow which may result from changes in the position of the heart or from increases in the muscle mass of one side of the heart as compared to that of the other side.

tions in environmental temperature were not encountered during the period in which the records were obtained. The factors of plane of nutrition and excitement due to changed surroundings may have exerted some influence on the rates observed in these cattle. It has been shown that heart rates are significantly higher when the plane of nutrition is raised to meet the requirements of heavy production (*5, 11*), and Rothberger and Winterberg (*21*) have demonstrated that sympathetic stimulation in dogs is accompanied by changes in the electrocardiogram similar to those observed by Lewis and Cotton during exercise. The effect of these factors, however, can hardly have been very great, although this possibility cannot be entirely disregarded.

TABLE 1.—*Heart rates of animals of different dairy breeds, based on an average of data from 3 leads and 3 monthly tracings; a summary of Fuller's data (11) is given for comparison*

Breed	Data for all age groups					Summary of Fuller's data				
	Animals	Heart rate (per minute)				Animals	Observations	Heart rate (per minute)		
		Minimum	Maximum	Mean	Standard error of mean			Minimum	Maximum	Mean
	Number	*Beats*	*Beats*	*Beats*	*Beats*	*Number*	*Number*	*Beats*	*Beats*	*Beats*
Jersey	23	48	83	65.9	1.74	9	356	46	84	62.7
Guernsey	22	49	98	72.7	2.65	7	317	48	72	59.8
Ayrshire	13	61	96	76.4	3.44	8	332	52	90	69.6
Brown Swiss	19	50	90	74.2	2.29					
Holstein	20	52	95	71.5	2.91	15	687	51	96	68.6
Total	97	48	98	71.6	1.22	39	1,692	46	96	65.9

TABLE 2.—*Heart rates of animals under 18 months of age compared to rates of animals over 18 months of age*

Breed	Age of animals			
	5 to 18 months		Over 18 months	
	Animals	Average heart rate	Animals	Average heart rate
	Number	*Beats per minute*	*Number*	*Beats per minute*
Jersey	5	71.4	18	64.3
Guernsey	5	90.0	17	67.6
Ayrshire	3	91.8	10	71.7
Brown Swiss	7	80.9	12	70.3
Holstein	5	82.0	15	67.7
Total	25	82.5	72	67.8

INTERVALS OF THE BOVINE ELECTROCARDIOGRAM

The three intervals, PR, QRS, and QT, were measured according to the method described under experimental procedure. A summary of the minimal, maximal, and mean measurements for the three intervals derived from the longest interval in any lead of the first monthly tracing, as well as the value for K (systolic index), is presented in table 3.

TABLE 3.—*The duration of the intervals and the values for K of the bovine electrocardiogram*

Breed	PR interval				QRS interval			
	Minimal	Maximal	Mean	Standard error of mean	Minimal	Maximal	Mean	Standard error of mean
	Second	*Second*	*Second*	*Second*	*Second*	*Second*	*Second*	*Second*
Jersey	0.10	0.22	0.18	0.0131	0.08	0.12	0.10	0.0128
Guernsey	.14	.28	.20	.0073	.08	.12	.10	.0035
Ayrshire	.17	.24	.21	.0005	.08	.11	.09	.0024
Brown Swiss	.14	.25	.18	.0067	.07	.12	.09	.0031
Holstein	.12	.30	.22	.0091	.06	.12	.09	.0037
Total all breeds	.10	.30	.19	.0002	.06	.12	.09	.0018

Breed	QT interval [1]				K (Bassett's formula)			
	Minimal	Maximal	Mean	Standard error of mean	Minimal	Maximal	Mean	Standard error of mean
	Second	*Second*	*Second*	*Second*				
Jersey	0.35	0.47	0.41	0.0061	0.40	0.47	0.425	0.0043
Guernsey	.31	.44	.38	.0095	.37	.48	.419	.0054
Ayrshire	.29	.42	.37	.0117	.35	.46	.407	.0097
Brown Swiss	.33	.47	.39	.0094	.37	.46	.424	.0059
Holstein	.29	.45	.38	.0077	.34	.46	.411	.0058
Total, all breeds	.29	.47	.39	.0039	.34	.48	.418	.0027

[1] The values used here are averages of all 3 leads in 3 tracings.

THE PR INTERVAL

The duration of PR as shown in table 3 ranged from 0.1 to 0.3 second, with an average duration of 0.19 second. Eighty-seven and six-tenths percent of the values were found in the range from 0.16 to 0.26 second. The spread here may in part be due to a tendency for PR to vary inversely with heart rate and to the fact that heart rates varied considerably among the various individuals.

In most instances the duration of PR was very nearly equal in all leads. Taking lead II as a standard, the values in leads I and III were usually within 0.01 second of this value. Occasionally the difference was as great as 0.02 second. Only very rarely was this difference exceeded. These variations agree rather closely with those found by Lewis and Gilder (*16*) in the human subject.

Variations in PR between the three serial electrocardiograms were likewise relatively minor. A variation of 0.01 second appearing in one or more leads occurred between the first and succeeding electrocardiograms of every individual. Less frequently there was a variation of 0.02 to 0.03 second. In eight animals the variation was 0.04 second, in one 0.05 second, and in two 0.06 second. These larger variations were due mainly to changes in the type of P wave in succeeding tracings. Quite often diphasic P was replaced in a succeeding tracing by monophasic P, or vice versa. This resulted in variations in the PR interval, and these were especially marked when there was variability in the initial effects of diphasic P. For instance, if the initial phase of diphasic P of one record should be absent in the suc-

ceeding electrocardiogram, giving rise to monophasic P, then the PR interval was usually shorter by a time interval equal to that of this absent initial phase. When monophasic P was replaced by diphasic P in the succeeding record, the interval was increased in an amount equal to that of the added phase. Similar variations with respect to initial effects of QRS were observed and these undoubtedly were in part responsible for variations in the PR interval.

TABLE 4.—*The duration of bovine electrocardiographic intervals, and the values for K at various levels of cardiac frequency*

Heart rate (beats per minute)	PR intervals				QRS intervals			
	Leads [2]	Minimal	Maximal	Mean	Leads [2]	Minimal	Maximal	Mean
	Number	*Second*	*Second*	*Second*	*Number*	*Second*	*Second*	*Second*
43 to 49	23	0.16	0.28	0.22	24	0.04	0.12	0.09
50 to 59	117	.15	.30	.22	130	.04	.12	.09
60 to 69	250	.10	.32	.20	265	.04	.12	.08
70 to 79	216	.08	.28	.19	237	.04	.12	.08
80 to 89	92	.12	.24	.17	103	.04	.12	.08
90 to 125 [3]	74	.11	.22	.15	96	.04	.11	.07

Heart rate (beats per minute)	QT intervals				K (Bazett's formula [1])			
	Leads [2]	Minimal	Maximal	Mean	Records	Minimal	Maximal	Mean
	Number	*Second*	*Second*	*Second*	*Number*			
43 to 49	22	0.40	0.50	0.45	8	0.372	0.434	0.400
50 to 59	129	.38	.52	.43	42	.373	.480	.420
60 to 69	263	.32	.48	.41	96	.354	.480	.424
70 to 79	232	.32	.44	.38	79	.357	.480	.424
80 to 89	101	.30	.44	.35	34	.353	.504	.421
90 to 95 [3]	92	.26	.38	.31	32	.326	.450	.392

[1] The value for K was calculated from the measurements in only 1 lead (generally lead II) of each electrocardiogram.

[2] The intervals in certain leads were not measureable because of the lack of distinctness of the waves bounding these intervals.

[3] Only 10 leads in this group showed a rate greater than 104 beats per minute.

The duration of the PR interval varied rather consistently with heart rate. Table 4 gives the data for the effect of heart rate on this and other intervals and for its effect on the value for K. It will be noted that the mean value for PR decreased regularly as the heart rate increased. The same general trend was seen in the minimal and maximal values found but was not so regular. Minor rate changes seldom altered the duration of PR noticeably, and there was considerable overlapping in the groups shown in table 4. It is evident from these data that the effect of heart rate on this interval must be taken into consideration before conclusions may be drawn concerning the significance of changes in PR. It was also noted that the PR interval was shorter in young than in mature cattle even though the heart rates were comparable.

Table 3 shows some slight differences between the breeds for the duration of the PR interval. The mean values ranged from 0.18 second for the Jerseys and Brown Swiss to 0.22 for the Holsteins. The variations here are not correlated with the different heart rates recorded for these two breeds (table 1), and because of the small size of each group the significance of these variations is questionable.

The QRS Interval

The duration of QRS ranged from 0.06 to 0.12 second with an average value of 0.09 second (table 3). The lower values of 0.08 second or less were found consistently in animals under 2 years of age. The distribution about the mean was quite close. Eighty-six and five-tenths percent of the values fell between 0.08 and 0.11 second, inclusive, and 95.8 percent were between 0.08 and 0.12 second. Probably one of the factors responsible for this was the fact that the duration of QRS did not vary with heart rate to such an extent as did PR and QT.

There was considerable variability in the duration of QRS between leads in any given electrocardiogram. In general, the smallest value was found in lead I and the largest in lead III. However, the difference between leads II and III rarely exceeded 0.01 second, although as much as 50 percent variation in the duration of QRS in lead I from that of leads II and III was observed.

Variations in interval length between serial electrocardiograms of individuals were relatively infrequent in leads II and III. When present they were usually within the range of 0.01 second. The greatest differences were seen in lead I, where deviations of 0.02 to 0.03 second were occasionally found. Since this is usually the unfavorable lead these results are not surprising, and are associated with monthly variations in the form of the low potential deflections.

Changes in length of this interval coincident with variations in heart rate were noted (table 4) in this series. The duration of QRS was less affected by variations in the heart rate than was that of PR and QT, but the decrease in the mean values for this interval as the heart rate increased was consistent. However, in view of the fact that the minimal and maximal values are practically identical for all rate levels, this variation in the mean may have no special significance. More extensive data on this point are needed.

No apparent breed differences were observed. The average duration of QRS for the entire series (0.09 second) is in close agreement with the average for the various breed groups.

The QT Interval

The duration of QT varied consistently with the heart rate in an inverse ratio, and, since a higher rate is common to younger animals, the values for QT in subjects under 2 years of age were usually less than those of mature animals. Table 4 shows the progressive decrease in duration of QT as the heart rate increases. The general trend of the data presented in this table is similar to the findings of Fridericia (*10*) and Pardee (*19*) in the normal human subject.

The QT interval ranged from a minimum of 0.29 to a maximum of 0.47 second. The average duration for the entire group was 0.39 second (table 3). Because of the inherent tendency of this measurement to vary inversely with the heart rate and since the rate varied in many instances from lead to lead as well as from month to month, the values which were the basis for table 3 are an average of the longest interval in each lead of the individual electrocardiograms. The distribution of the values about the mean was approximately the same as for the PR interval. Eighty-seven and six-tenths percent

of the values fell in the range of 0.34 to 0.46 second, a spread of 0.12 second as compared to 0.10 second for the PR interval. The greater spread of the distribution of the QT values as compared to those for QRS was mainly due to the fact that QT varied with heart rate to a greater extent than did QRS and consequently was affected to a greater degree by the higher rates found in the younger cattle.

Variations in the duration of QT between leads while not extreme were more common with this interval than with any other. Taking lead II as a standard, a plus-or-minus difference of 0.01 to 0.02 second was very common. Variations greater than this were infrequent. The maximum variation was 0.06 second. Generally there was a tendency for the interval in leads II and III to be nearly equal, with lead I showing a slightly smaller value.

Monthly variations were even more pronounced than the differences observed between the leads. In general they were closely associated with variations in heart rate, there being an increase in duration of QT with a decrease in heart rate, and vice versa. They were, however, accounted for partly by the fact that the type of T wave varied from month to month in the same individual. A comparable variability in the type of P wave with similar effects has been noted previously (p. 65). Variations extreme enough to cause a marked difference in QT time are relatively infrequent provided the longest interval in any lead be chosen for measurement. Furthermore, any slight influence which variations in T may exert tend to be obscured by the interval changes due to differences in rate. This phase of electrocardiography in the human subject has been statistically treated by Adams (*1*).

No significant breed differences were detected. The QT interval in the Jersey group which showed the lowest average heart rate of any of the breeds exceeded the average for all breeds by 0.02 second, while in the Ayrshire group the interval was shorter by 0.02 second than the average for the group as a whole. The average for the other breeds was very close to the average for the entire series. The number of animals and the variation from the mean are too small to warrant definite conclusions regarding any possible differences that may exist between the Jerseys and Ayrshires on the one hand and the Holsteins, Brown Swiss, and Guernseys on the other.

It will be noted that the duration of this interval, as well as that of PR and QRS, was quite similar to the duration of the same intervals of the human electrocargiogram. This is quite surprising in view of the greater mass and the greater thickness of the ventricular walls of the bovine heart as compared to the human heart. The observations of Cardwell and Abramson (*6*) and of Alfredson and Sykes (*2*) suggest that the relatively short interval length of the bovine electrocardiogram is due to differences in the distribution of the Purkinje network in the bovine heart as compared to that of the human heart.

The Systolic Index

In calculating K (systolic index), lead II was usually the most favorable lead to use since the waves, QRS and T were generally more distinct in this lead than in leads I or III. However, whenever the complexes of leads I or III were more suitable than those of lead II, K was calculated from one of these leads.

The value for K ranged from 0.34 to 0.48 with an average of 0.418 (table 3). Although 52 of the 97 animals showed a value for K within the range 0.41 to 0.43, the range 0.34 to 0.48 for the entire group is quite wide. When the values for K in the individual electrocardiograms were averaged and the values so obtained grouped according to the age of the animals, it was apparent that there was a progressive increase in the value for K up to 2 years of age, at which point K became relatively constant. These data, summarized in table 5, show that the value for K is not constant for all age groups, particularly for younger cattle. Moreover, table 4 shows that, at rates below 50, and especially above 90, beats per minute, K varies with rate to some extent. Since the higher rates are common to younger cattle (table 2), the lower values for K in young cattle and the rather wide range for K shown in table 3 would seem to be largely due to the fact that Bazett's formula failed to make an adequate correction at the higher rate levels found in some of these cattle. More extensive data would be desirable on these points.

Minor variations in the systolic index were noted from month to month. These variations could not always be correlated with changes in rate. Using the values in the second monthly tracing as a basis for comparison, the plus-or-minus differences from the first and third monthly tracing were obtained, as shown in the following tabulation:

Range of variability	*Number of observations*
Equal in all three records [1]	6
Equal in either first or third record	18
Inequality in all three records:	
±0.001–0.009	51
±0.010–0.019	59
±0.020–0.029	27
±0.030–0.039	20
±0.040–0.049	3
±0.050–0.059	3
±0.060–0.069	1

[1] This agreement is within 0.001.

There were no breed differences, the values for K being relatively constant for all breeds and close to the average for the entire group in all cases.

TABLE 5.—*Minimum, maximum, and mean values for K (Bazett's formula (4)) at various age levels*

Age group	Number of animals	K (Bazett's formula)		
		Minimum	Maximum	Mean
5 to 8 months	3	0.34	0.40	0.377
8 to 10 months	6	.36	.41	.390
10 months to 1 year	8	.37	.42	.398
1 to 1½ years	7	.35	.44	.408
1½ to 2 years	13	.38	.47	.415
2 to 3 years	12	.38	.46	.429
3 to 4 years	17	.39	.46	.428
4 to 5 years	10	.39	.47	.426
5 to 6 years	9	.40	.45	.433
6 to 8 years	5	.41	.44	.424
8 to 10 years	3	.43	.48	.450
10 to 12 years	4	.39	.42	.410

FORM AND OCCURRENCE OF BOVINE ELECTROCARDIOGRAPHIC DEFLECTIONS

THE P WAVE

The P wave, in common with all the deflections composing the bovine electrocardiogram, showed characteristics differing materially from the P wave seen in human tracings. These differences were largely increases in the incidence of diphasic and extremely low potential waves or, more rarely, complete absence of the deflection. Monophasic waves consisting of an upward or positive deflection and diphasic waves with a positive net area[8] were the two common types of waves. In two or three instances negative monophasic waves were encountered. The sequence of events, where diphasic waves were present, was usually an initial quick downward deflection followed by a much slower upward phase. Only four individuals showed diphasic P with a positive to negative sequence. True notching of P, such as was found by Kahn (*12*) in the horse and to a lesser extent by Nörr (*18*) in cattle, when employing the single regio-apicis:regio-praescapularis lead, did not occur in a single instance in this series.

Monophasic waves were most common in lead III; they appeared less frequently in leads I and II. Diphasic waves were most numerous in leads I and II and appeared only 8 times in lead III. Occasionally (10 times in lead I and 9 times in lead III out of a total of 873 leads) P was totally absent or when present was of such extremely low potential that its form could not be accurately determined. The deflection was always present in lead II.

TABLE 6.—*Summary of the occurence of various combinations of P waves with respect to leads in the first monthly tracings* [1]

Combinations			Observations by breeds					
Lead I	Lead II	Lead III	Jersey	Guernsey	Ayrshire	Brown Swiss	Holstein	Totals
D	D	D	0	0	2	1	4	7
D	D	+	8	10	3	5	8	34
D	+	+	7	2	3	1	3	16
+	D	D	1	0	0	0	0	1
+	D	+	1	1	1	2	0	5
−	D	+	0	0	0	0	2	2
+	+	+	3	2	0	2	2	9
+	+	−	0	0	0	1	0	1
+	D	NM	0	1	0	0	0	1
NM	+	+	1	0	0	0	0	1
NM	D	+	2	5	0	0	0	7
D	D	NM	0	1	2	3	0	6
NM	+	+	0	0	1	1	1	3
+	+	NM	0	0	1	2	0	3
M	+	+	0	0	0	1	0	1
Total			23	22	13	19	20	97

[1] Key: +=Upward monophasic deflection; −= downward monophasic deflection; D=diphasic wave; NM=absent, nonmeasurable, or nondeterminable wave; M=M-shaped complex with positive net area.

The combinations of the various forms of the P wave which were found in the three leads of the electrocardiograms are summarized in table 6. In compiling this table only the first tracing obtained on each animal was considered. Of the 64 possible combinations of positive and negative monophasic, diphasic, and nondeterminable

[8] In waves of the diphasic type or in QRS complexes of the vibratory type, a negative net area indicates that the area encompassed by the negative deflection is greater than a similar area enclosed by the positive phase. A positive net area indicates the opposite of this.

waves, only 15 occurred. Of these the DD+[9] combination was seen most frequently (34 times). The sum of the instances in which DD+, D++, DDD, and +++ combinations appeared, accounted for 68 percent of the records. When monophasic P occurred in only one lead of an electrocardiogram, it almost invariably appeared in lead III. Its presence in any lead other than III was always coincident with its appearance in at least two leads. When diphasic P appeared in only one lead, it was usually found in either lead I or II. It appeared more frequently, however, in two or more leads, the most usual occurrence being in both leads I and II.

The QRS Group and the Classification of the Bovine Electrocardiogram

The occurrence of the individual deflections of QRS was as follows: Q appeared 59 times in lead I, 78 times in lead II, and 68 times in lead III; R appeared 76 times in lead I, 97 times in lead II, and 97 times in lead III; S was found 28 times in lead I, 8 times in lead II, and 20 times in lead III; R′ was found twice in lead I, 5 times in lead II, and 14 times in lead III. The percentage occurrence of these deflections in the various leads is presented in table 7. The above data were obtained from the first electrocardiogram of each animal. Table 7 indicates the invariable occurrence of R in leads II and III, the great frequency of Q in all leads, and the relative infrequency of S and R′.

The various representative types of QRS encountered in this study are presented in figure 2. The arrangement is purely arbitrary. Beginning with R-wave predominance, the progression is through varying types of multiphase complexes to negative (Q-wave) predominance and finally representative unfavorable low-potential QRS types.

Types 1 to 6 include those complexes showing definite R-wave predominance. In types 1 and 2 both limbs of the R wave are similar, varying from a quick fine upstroke and downstroke to complexes in which both limbs of this deflection are coarsened. Type 1 occurred in only one individual. Types 3 and 4 are characterized by a fine quick upstroke of R with slurring or coarsening of the descending limb, which may be complete (type 3) or merely confined to its terminal portion (type 4). Two similar forms of QRS are grouped under type 5. Type 5, *a*, shows notching of the downstroke at the point where the terminal portion becomes coarsened. With a lowering of the potential of the initial spike, forked or M-shaped complexes such as 5, *b*, occur. In type 6 the descending limb of the initial spike crosses the isoelectric level, giving rise to a second positive QRS deflection or R′ wave. From type 6, the forms pass through various stages of M-shaped complexes in which the notching may (type 8) or may not (type 7) cross the isoelectric level. Types 9 to 12, inclusive, exhibit downwardly directed initial effects of QRS. The complexes as a whole are W-shaped vibratory or truly W-shaped (types 9 and 10). In many of these a second positive QRS deflection, R′, was present. Type 11 consists of diphasic (QR) complexes. The potential of the positive and negative phases of this type was sufficiently large to indicate considerable rotation of the cardiac electrical

[9] The symbol DD+ means that the deflection was diphasic in leads I and II and monophasic in lead III. Similarly +++ indicates a positive monophasic wave in all three leads, etc.

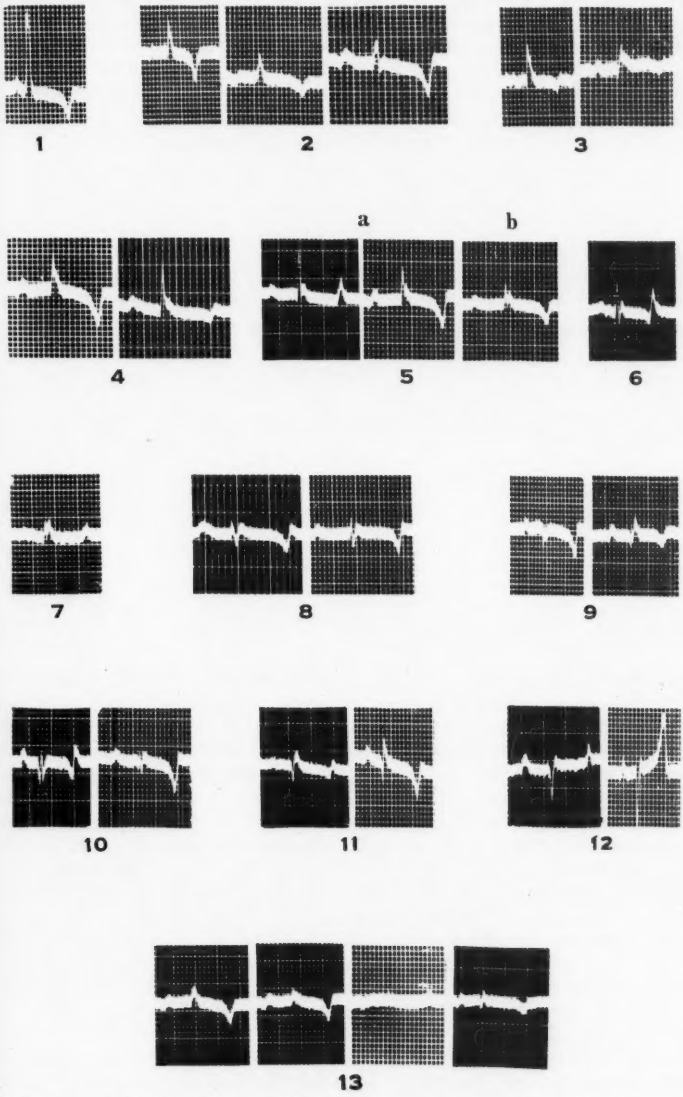

FIGURE 2.—Various types of bovine electrocardiographic complexes arranged according to form of the QRS group.

axis during systole. Type 12 is characterized by Q predominance. Under type 13 are grouped all extremely unfavorable low potential QRS complexes. Many of these resemble other types, but are not sufficiently well defined to justify classification as such. This form

occurred most frequently in lead I. The occurrence of the various types in the three leads is presented in table 8. The predominance of types 2, 4, 11, 12, and 13 will be noted.

TABLE 7.—*Occurrence of the various deflections composing QRS in the three leads*

Lead No.	Occurrence of deflection—			
	Q	R	S	R′
	Percent	*Percent*	*Percent*	*Percent*
I	60	78	29	2
II	80	100	8	5
III	70	100	21	14

TABLE 8.—*Distribution of the QRS types, represented in figure 2, in the three serial electrocardiograms* [1]

Lead No. (all breeds)	QRS type													Total leads
	1	2	3	4	5		6	7	8	9	10	11	12	13	
					a	b									
I	3	57	6	3	0	0	0	2	1	12	3	25	63	116	291
II	3	11	30	89	16	10	0	8	6	15	6	65	21	11	291
III	3	5	11	75	24	26	20	19	19	17	16	24	11	21	291
Total	9	73	47	167	40	36	20	29	26	44	25	114	95	148	873

[1] Since there were 3 serial electrocardiograms for each of the 97 animals represented, the data for this table were obtained from a total of 291 records (873 leads).

In most instances QRS did not closely resemble QRS in the electrocardiograms of normal human subjects. This dissimilarity is possibly due to one or both of two factors. (1) The electrical axis of the bovine heart lies in a plane relative to the three leads which is considerably different from the plane of the electrical axis of the human heart in respect to the same three leads. This is due to the fact that the position of the heart in the chest cavity is different in the two species, and since the form of QRS depends in part at least on the relation of the electrical axis of the heart to the leads, this anatomical difference could partly account for the dissimilarity of QRS in the bovine and human electrocardiogram. (2) It seems altogether probable that the spread of the impulse in the bovine heart may be different from that in man. Cardwell and Abramson (*6*) have shown that the distribution of the Purkinje network is different in the two species and the writers' observations on bundle branch block (*2*) tend to confirm this finding.[10]

The various types of QRS complexes seen in figure 2 occurred in numerous different combinations in the individual electrocardiograms. In fact they were so numerous that a separation of the electrocardiograms into classes which would show similar combinations of QRS

[10] The impulse which activates the heart travels over a rather definite pathway of specialized conducting tissue. This tissue consists of (1) the bundle of His and its two bundle branches, one of which is distributed to each ventricle, and (2) the Purkinje network which consists of branches from the two bundles and conveys the impulse to the ventricular muscle. When the conducting system is damaged experimentally (bundle branch block) marked changes occur in the electrocardiogram of the subject as a result of the altered conduction pathway.

types in the three leads resulted in such a conglomeration of groups, each consisting of only a few electrocardiograms, that this method became altogether unsuitable as a basis for a workable classification of the bovine electrocardiogram. However, when the type of QRS in lead II alone is considered, the electrocardiograms separate rather distinctly into four main groups. These four groups and the combinations of QRS types which occurred within these groups are shown in figure 3.

TYPE I

The type I group is characterized by R-wave predominance in lead II. All variations of R waves (fig. 2, types 1 to 6) were seen, although types 4, 5, and 6 predominated. This main group may be separated into two divisions, the basis for segregation being the occurrence of R waves in both leads I and II or in leads II and III. Generally speaking, the type of R in lead I or III is similar to that in lead II, although notching may occur in one lead without appearing in the other. The potential of R in leads I and II, or II and III, is usually greater in these two leads than in the other lead when R occurs in that lead.

Type I, A.—This type is characterized by R-wave predominance in leads I and II (fig. 3, Nos. 1, 2, and 3). Lead III shows mainly M-shaped QRS complexes (fig. 2, type 7), although QR type complexes (fig. 2, type 11) and complexes with R predominance (fig. 2, type 5, *b*) may appear. These are mainly of positive net area and of small potential.

Type I, *B.*—This type is characterized by R-wave predominance in leads II and III. QRS in lead I may be represented by R (fig. 3, No. 7), QR (fig. 3, No. 6), or Q waves (fig. 3, No. 5). Vibratory M- or W-shaped complexes (fig. 3, Nos. 4 and 8) are also common. These complexes are generally of small potential. The electrocardiogram of one animal which was included in this group consisted of R waves of type 1 (fig. 2) in all three leads. This was the only electrocardiogram of this type which was encountered.

TYPE II

The type II group as a whole is characterized by a diphasic QRS complex (fig. 3, type 11) in lead II. Generally the complexes in leads I and III are of opposite direction, a positive net area of QRS in lead I usually being accompanied by a negative net area of QRS in lead III and vice versa.

The most common combination in leads I and III is Q (fig. 2, type 12) in lead I accompanied by R of type 3, 4, 5, or 6 (fig. 2) in lead III. Twelve of 22 electrocardiograms in this group were of this type (fig. 3, Nos. 9 and 10). The remaining 10 electrocardiograms show considerable variation in leads I and III. Four showed Q waves in lead I accompanied by diphasic QRS in lead III (fig. 3, No. 11). Four were of positive net area in lead I and showed diphasic or W-shaped QRS in lead III (fig. 3, Nos. 12 and 13). Of the two remaining electrocardiograms, one displayed a diphasic (QR) type of QRS in lead I and an M-shaped QRS in lead III (fig. 3, No. 14) and the other a W-shaped QRS in lead I and an M-shaped QRS in lead III (fig. 3, No. 15).

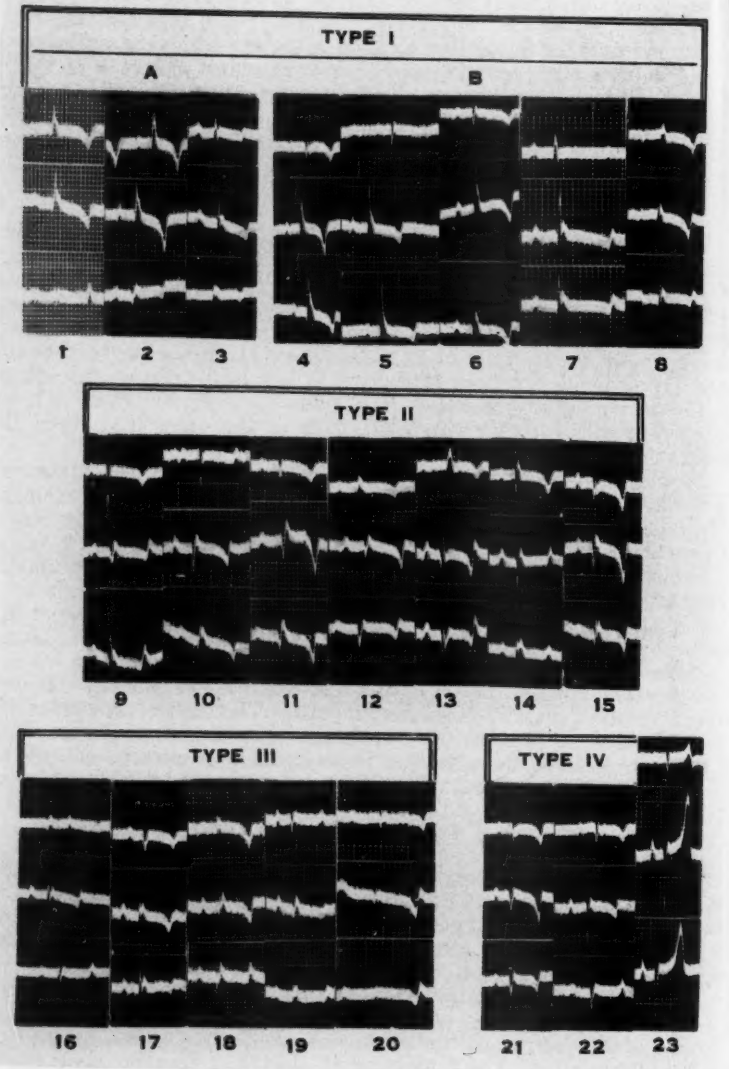

FIGURE 3.—Types of the bovine electrocardiogram. The Arabic numbers indicate variations of the basic types which were found to occur. The arrangement of the leads is I, II, and III from top to bottom.

TYPE III

The type III group includes all electrocardiograms which are of uniformly small potential in all leads. While some resemblance to electrocardiograms of the preceding two groups may be seen in a few instances, considerable variation from month to month is encountered,

and the potential of these complexes is so small as compared to the potential of the principal deflections in types I and II that inclusion in any other group is not justified.

Within this group M- and W-shaped QRS complexes (fig. 2, types 8, 9, and 10) predominate in lead II. The most commonly occuring combinations are: (1) M-shaped QRS in lead II and diphasic or M-shaped QRS in lead III with R in lead I (fig. 3, No. 16); (2) M-shape complexes in leads II and III (fig. 3, No. 17); (3) M-shaped in lead II and W-shaped in lead III with R in lead I; (4) W-shaped in lead II and M-shaped in lead III; and (5) W-shaped in leads II and III (fig. 3, No. 18). Six electrocardiograms placed in this group rather closely resemble those of type II in that the complex in lead II tends to be diphasic. However, since there was some variation in successive tracings tending toward M- and W-shaped complexes in lead II and since the potential was small in all three leads, they have been included in this group (fig. 3, No. 19). In a few instances the potential of QRS in all three leads was so small and the complexes so poorly defined that QRS was practically nonexistent (fig. 3, No. 20).

TYPE IV

The main characteristic of the type IV group, composed of 6 of the 97 animals, is the predominance of Q (fig. 2, type 12) in at least two leads of the electrocardiogram. Four of these show Q predominance in leads II and III accompanied by Q of small potential in lead I (fig. 3, Nos. 21, 22, and 23). One shows effects similar to the above in leads II and III with diphasic QR in lead I, and one consists of Q in leads I and II with poorly defined complexes showing a negative net area in lead III.

SUMMARY OF TYPES I TO IV

The electrocardiograms of 10 of the 97 animals classify under type I, *A;* 42 under type I, *B;* 22 under type II; 17 under type III; and only 6 under type IV. Electrocardiograms which classify in types I and II seem to be typical of a large proportion of the cattle (76.3 percent) used in this study, while a considerably smaller proportion fall in types III and IV. While minor variations occur within each group, the major divisions are generally quite distinct, and it is suggested that these groups be used, at least for the present, as a basis for the classification of the bovine electrocardiogram.

The T Wave

The deflection T, like the P wave, presented characteristics rarely encountered in human tracings. Diphasic T waves occurred much more frequently than they do in the human subject. They were usually present in one or more leads of most electrocardiograms. Monophasic waves resemble those seen in the human subject except that the summit tends to be more pointed. A great proportion of monophasic T waves, especially in leads I and II, were negative while those in lead III were mainly positive. One limb was often more quickly executed and therefore finer in outline than the other. Diphasic waves were largely characterized by slow broad initial and terminal deflections, the movement of the string during change in direction of potential being usually quite rapid. The sequence of events, as with diphasic P, was, with two exceptions, an initial negative phase followed

by a positive phase. The various types encountered are shown in figure 3. Triphasic T was seen only once and was absent in succeeding electrocardiograms of this one individual. Low potential undeterminable T waves were present only eight times and complete absence of T was never observed.

Monophasic T was most frequently seen in lead I (79 times) and appeared about equally in leads II and III (27 and 29 times, respectively). Diphasic T waves were most numerous in leads II and III and showed a negative net area in 107 of the 146 leads in which this type of deflection occurred.

Only 19 of the 64 possible combinations of the various types of T in the three leads of the individual electrocardiograms occurred. The three most frequent combinations were —DD (37 times), — —D (17 times), —D+ (13 times). This accounts for 69 percent of the 97 animals constituting this series. When monophasic T occurred in only one lead it was always in lead I or III, never in II. Its occurrence in II was always coincident with monophasic T_1, T_3, or both, simultaneous appearance in leads I and II being the most frequent combination. When diphasic T occurred in only one lead it appeared most frequently either in lead II or III. When present in more than

TABLE 9.—*Summary of the occurrence of various combinations of T waves with respect to leads in the first monthly tracings* [1]

Combinations			Observations by breeds					
Lead I	Lead II	Lead III	Jersey	Guernsey	Ayrshire	Brown Swiss	Holstein	Totals
D	D	D	2	1	1	1	0	5
D	D	+	1	0	2	0	0	3
D	D	−	1	1	0	0	0	2
D	+	+	0	3	0	0	0	3
+	D	D	1	0	0	0	0	1
−	D	D	12	7	3	6	9	37
−	−	D	2	2	0	5	8	17
+	D	−	1	0	0	0	0	1
−	D	+	0	4	4	3	2	13
−	D	−	0	0	1	0	0	1
+	−	−	0	1	0	0	0	1
−	+	+	1	0	0	0	0	1
−	−	+	0	1	0	2	0	3
+	D	ND	1	0	0	0	0	1
ND	D	D	0	1	0	1	1	3
ND	D	−	1	0	0	0	0	1
D	D	ND	0	0	1	0	0	1
−	−	ND	0	0	1	1	0	2
−	D	W [2]	0	1	0	0	0	1
Total			23	22	13	19	20	97

[1] Key: +=Upward monophasic deflection; −=downward monophasic deflection; D=diphasic wave; ND=nonmeasurable or nondeterminable wave; W=W-shaped complex.
[2] Triphasic wave with slightly positive net area.

one lead the most frequent combination was in leads II and III. Table 9 summarizes the occurrence of the various combinations of T which were encountered.

THE POTENTIAL OF THE ELECTROCARDIOGRAPHIC DEFLECTIONS

As may be judged from previous discussion there was great variation in the potential of the several deflections. Table 10 shows the minimal, maximal, and mean values of the potential for each wave as they were observed in the three leads.

TABLE 10.—*The potential of the deflection in the 3 leads*

Deflection	Lead	Minimal[1]	Maximal	Mean	Deflection	Lead	Minimal[1]	Maximal	Mean
		Millivolt	*Millivolts*	*Millivolt*			*Millivolt*	*Millivolts*	*Millivolt*
P	I	0.03	0.12	0.06	S	I	0.03	0.20	0.06
	II	.03	.18	.10		II	.03	.10	07
	III	.03	.22	.07		III	.03	.25	.12
Q	I	.03	.60	.23	R′	I	.10	.20	.15
	II	.03	1.00	.16		II	.03	.10	.11
	III	.03	.90	.11		III	.03	.20	.10
R	I	.03	.70	.16	T	I	.03	.60	.20
	II	.03	2.60	.37		II	.03	1.10	.31
	III	.03	.50	.35		III	.03	.90	0.18

[1] These values do not represent the absolute minimum since any deflection with a potential smaller than 0.03 millivolt cannot be accurately measured and was arbitrarily given this value.

The minimal values were equal in all three leads for every deflection with the exception of R′ in one instance (lead I). The largest deflections of P were found in leads II and III. Lead II, however, showed the greatest mean value for this deflection. Nondeterminable P waves were frequently found. These occurred 28 times in lead I and 17 times in lead III, and therefore the values shown in table 10 do not represent the absolute minimum in these leads. No other deflection showed such a high incidence of nondeterminable waves. Maximum values for Q occurred in leads II and III, and the highest mean value was found in lead I. In the case of R the greatest deflection was found in lead II, although the mean values for R were about equal in leads II and III. The potential of S was uniformly small as compared to the other deflections of QRS. The greatest value observed as well as the greatest mean value was found in lead III. While R′ showed maximal values of 0.20 millivolt in leads I and III and a mean value of 0.15 millivolt in lead I, the significance of these values and of the deflection itself is doubtful. Since R′ occurs infrequently and is then uniformly of low potential, and since it is usually associated with bizarre, splintered R waves and is subject to considerable variation in successive tracings, it could more justifiably be disregarded. According to Katz and associate[s] (*14*), this is the usual practice when R′ occurs in lead III of human electrocardiograms. The largest potential of T was found in leads II and III. Lead II showed the largest mean value for the potential of this deflection.

Since it is recognized that the potential of the QRS deflection may be correlated with the nutritional state of the heart muscle in the human subject, and since the largest deflection seen in any lead of the eletrocardiogram is used as a basis for this comparison, the greatest potential of the various deflections in the first record obtained on each individual has been determined. These values, shown in table 11, are not comparable to those in table 10 since the values there shown include the potential for every deflection observed. With the exception of the P wave the values showed considerable variation. Q was absent in all leads in 10 instances, S was absent in the electrocardiograms of 54 animals, and R′ was absent in 69 records. The occurrence of low potential QRS in all three leads in some electrocardiograms of apparently normal cattle (see fig. 3, type III), however, indicates that the size of the potential of QRS deflections as an index of the nutritional state of the heart muscle may not be applicable to cattle as it is in the human subject.

The data given above offer no opportunity for broad generalizations concerning the potential of the various deflections beyond the fact that Q and S were generally of smaller potential than R and T and were absent in all leads of the electrocardiograms in a fairly large percentage of the animals studied, whereas R and T were always present.

TABLE 11.—*The potential of the deflections of the bovine electrocardiogram when only the largest observed deflections were considered*

Breed	P				Q				R			
	Minimal	Maximal	Mean	Standard error of mean	Minimal	Maximal	Mean	Standard error of mean	Minimal	Maximal	Mean	Standard error of mean
	Millivolt	*Millivolt*	*Millivolt*		*Millivolt*	*Millivolt*	*Millivolt*		*Millivolt*	*Millivolt*	*Millivolt*	
Jersey	0.10	0.20	0.13	0.0049	0.0	0.60	0.22	0.0370	0.10	2.60	0.62	0.1021
Guernsey	.05	.22	.11	.0065	.03	1.00	.30	.0486	.10	.65	.34	.0433
Ayrshire	.03	.13	.08	.0076	.0	.40	.15	.0333	.20	1.00	.39	.0602
Brown Swiss	.05	.18	.10	.0072	.03	.80	.25	.0461	.10	.95	.36	.0504
Holstein	.03	.12	.09	.0050	.0	.40	.09	.0278	.10	1.10	.39	.0532
Total (all breeds)	0.03	0.22	0.11	0.0031	0	1.00	0.21	0.0197	0.10	2.60	.43	0.0336

	S				R′				T			
Jersey	0	0.50	0.04	0.0216	0	0.20	0.02	0.0114	0.15	0.70	0.32	0.0257
Guernsey	0	.20	.04	.0126	0	.20	.02	.0102	.10	.90	.24	.0351
Ayrshire	0	.30	.07	.0223	0	.20	.06	.0194	.15	.70	.33	.0419
Brown Swiss	0	.10	.01	.0058	0	.15	.01	.0085	.18	.60	.35	.0265
Holstein	0	.30	.07	.0208	0	.20	.06	.0134	.15	.70	.43	.0334
Total (all breeds)	0	0.50	0.04	0.0081	0	0.20	0.03	0.0057	0.10	0.90	0.33	0.0161

VARIATIONS IN THE DEFLECTIONS OF THE BOVINE ELECTROCARDIOGRAM IN SUCCESSIVE TRACINGS

Pronounced variations in the deflections from month to month were not frequent. This was particularly true of the deflections of the QRS complex. Minor changes, however, were rather numerous. Figure 4 illustrates the variations encountered.

Monthly changes in P occurred more frequently in the low potential waves; this was especially true in lead I and to a lesser extent in lead III. The principal variation was in potential. P may be totally absent one month and present another (fig. 4, *a*, *b*, *e*, and *i*). A similar variation of P may occur within a lead (fig. 4, lead III, *k*). In one subject (fig 4, lead I, *c*) there was a reversal of potential. The other major variation in P was a change in the form of this wave. Diphasic P waves with positive or negative net area often changed to monophasic P in a subsequent tracing. Similarly, monophasic P frequently changed to diphasic P. These changes are illustrated in figure 4, *c*, *g*, and *h*.

Monthly changes of the deflections composing the QRS complex were not marked. Minor variability in low potential Q and S waves, in the form of QRS characterized by diphasic, M-, or W-shaped complexes, and in R waves was frequently seen. Various degrees of coarsening and notching of R in successive tracings were observed (fig. 4, *a*, *b*, *c*, *d*). The changes showed no definite trend, there being no correlation between notching in one tracing and coarsening of the downstroke in the others. Subjects *e*, *f*, *g*, *h*, and *i* (fig. 4)

show, in addition to R and Q wave changes, variations in the vibratory type of QRS which were so frequently encountered. The change here was almost invariably an increase in the excursion of the descending limb of the first spike which was often sufficiently great to cross

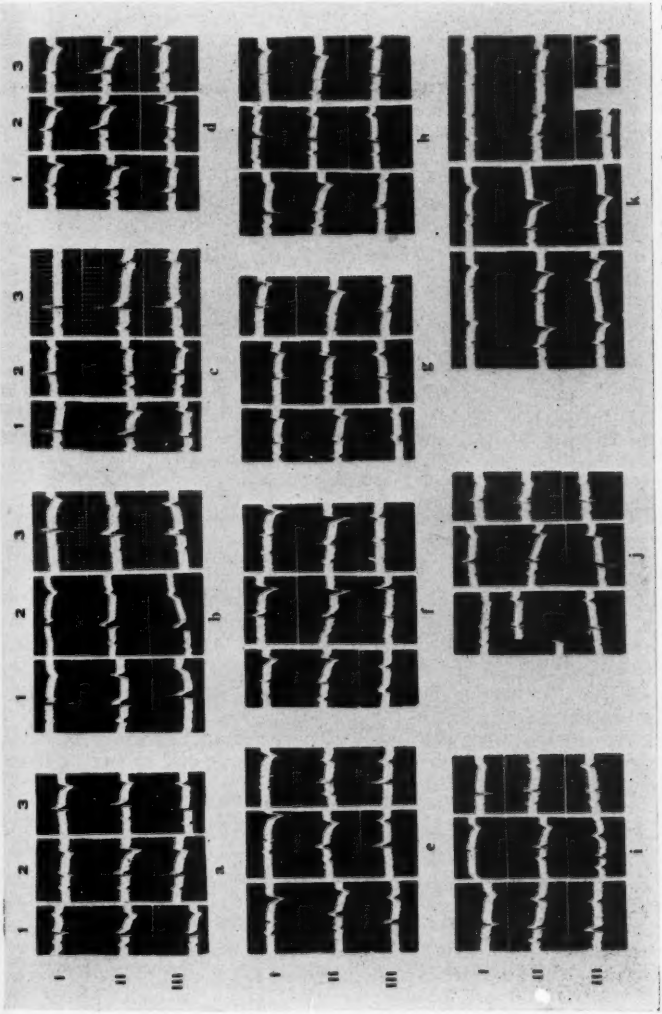

FIGURE 4.—Monthly variations in serial electrocardiograms in bovines. The Arabic numbers 1, 2, and 3 indicate first, second, and third monthly tracings, respectively; the roman numerals to the left of the figure indicate the derivation or lead; and the letters *a*, *b*, *c*, etc., designate individual animals. The variation within lead III of the third monthly tracing in subject *k* occurred during the recording of the lead.

the isoelectric level. Similar changes in the ascending limb of the initial spike occurred where vibratory QRS complexes were of negative net area.

Monthly variations in the T wave were more striking than those of any other deflection, although no very definite trend was noted.

These changes were not associated, as was the case with P, with low potential waves. The principal variation seen was from a diphasic to a positive or negative monophasic wave or vice versa, and this change was not always in the direction of the predominant wave of diphasic T in previous tracings. Figure 4, *a*, *e*, *h*, *i*, and *k*, illustrates typical variations. In view of the foregoing observations, it is apparent that variations in the T wave in succeeding tracings do not have the same significance as do changes in T in the human subject.

No variations were found to be correlated with stage of pregnancy, heat, or stage of lactation. Nörr (*18*) likewise was unable to detect any differences in the electrocardiogram of cattle following parturition. However, it would be desirable to obtain records over the whole period of gestation and lactation in order to determine the effect of these factors more accurately.

The electrocardiograms have been examined for changes within leads. Marked variations were noted in only a few instances. Changes accompanying the various phases of respiration such as have been described in the human subject and by Nörr (*18*) in cattle were not observed in this series. Slight differences in the potential of QRS (0.05 to 0.10 millivolt) were frequently observed. Occasionally variations in potential within a lead were accompanied by alterations in the type of complex (fig. 5, Nos. 1 and 2). When periods of restlessness occurred while the record was being taken, distinct changes in the electrocardiogram were noted in a few instances in that portion of the record subsequently obtained. These are shown in figure 5, Nos. 3 and 4.

THE ELECTRICAL AXIS OF QRS IN THE BOVINE HEART

The electrical axis of QRS was in the range from +30° to +90° for 50 percent of the animals. The electrical axis in another 17 percent was in the +91° to +170° range. Only 17 percent were grouped in the −30° to −170° range, while 6 percent showed the extreme deviation of 180°. No values were found between 0° and +30° or between 0° and −30°. These data are shown in table 12.

The electrocardiograms of nine animals were of such a nature that it was impossible to determine the electrical axis even approximately. These electrocardiograms were generally characterized by unfavorable QRS types (diphasic and vibratory) of small potential in two or more leads (fig. 3, nos. 17 and 18).

TABLE 12.—*The occurrence of the various electrical axes of QRS*

Breed	Range					
	+30° to +90°	+91° to +170°	−30° to −90°	−91° to −170°	180°	Non-determinable
Jersey	13	5	0	3	2	0
Guernsey	7	5	4	3	2	1
Ayrshire	6	2	1	0	1	3
Brown Swiss	8	5	0	3	1	2
Holstein	14	0	2	1	0	3
Total	48	17	7	10	6	9

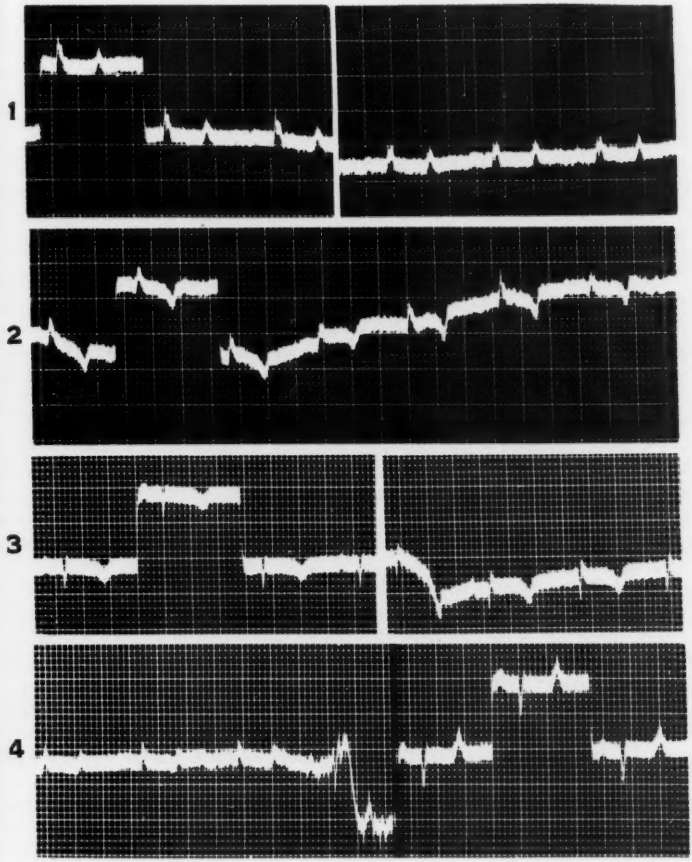

FIGURE 5.—Intralead variations in four bovine electrocardiograms. In order to include significant portions of the records, a few complexes were cut from the middle of nos. 1 and 3.

The electrical axis showed some variation in succeeding monthly tracings. Differences sufficiently great to be detectable by the method of analysis employed were seen in 26 animals, but these were not extreme. However, since changes did occur, they must be taken into account in drawing conclusions from experimental procedures which involve repeated records. The variations in electrical axis which occurred in successive tracings are ascribed to the relatively greater mobility of the bovine heart as compared to the human heart. Katz and his associates (*13*) have observed similar effects in the dog, in which the heart likewise shows greater mobility that it does in man. Table 13 shows the incidence and the distribution of the axis variations from that present in the first monthly tracings.

Because of the methods employed and the general unfavorableness of some tracings, the data presented in this section are only of the most general nature. More detailed study concerning the spread of the impulse in the bovine heart and other factors which may determine the form of QRS in this species is essential before definite conclusions can be drawn.

TABLE 13.—*The occurrence of axis variations in successive tracings* [1]

Breed	Variation							
	10°	20°	30°	40°	45°	60°	80°	Total
Jersey	0	0	2	1	0	0	0	3
Guernsey	3	1	3	0	1	0	1	9
Ayrshire	2	0	1	0	0	1	0	4
Brown Swiss	3	2	2	0	0	0	0	7
Holstein	0	1	2	0	0	0	0	3
All breeds	8	4	10	1	1	1	1	26

[1] In compiling these data the first of the 3 records was used as a standard.

SUMMARY AND CONCLUSIONS

Three serial electrocardiograms, taken approximately 1 month apart, were obtained on each of 97 normal dairy cattle. The data derived from an analysis of these records may be briefly summarized as follows:

(1) The heart rate of the cattle as a whole averaged 71.6 beats per minute. The rate for animals up to 1½ years of age, however, was significantly greater than this (82.5), while for animals over 1½ years of age the rate was lower (67.8). These figures probably represent fairly closely the average rate for normal dairy cattle.

(2) The duration of the PR interval ranged from a minimum of 0.10 second to a maximum of 0.30 second, with an average of 0.19 second. The QRS interval averaged 0.09 second. The distribution of the values about the mean for this interval was quite close, the minimum and maximum duration being 0.06 and 0.12 second, respectively. The QT interval ranged from a minimum of 0.29 second to a maximum of 0.47 second with an average duration of 0.39 second. The duration of QT and, to a lesser extent, of PR was inversely proportional to the heart rate. The QRS interval also varied with heart rate but this effect was not as pronounced nor as constant as with the PR and QT intervals.

Variations in interval length which were not correlated with rate occurred frequently in the same individual. The most pronounced differences were seen in the QT interval. Smaller variations were found in the PR interval and still smaller in the QRS. Many of these changes were associated with variations in the form of the various deflections and with poorly defined deflections in certain leads, especially lead I.

(3) The systolic index as determined by Bazett's formula ranged from 0.34 to 0.48 with an average of 0.418. Individual monthly variations of 0.001 to 0.030 were not uncommon. The index was relatively constant in the electrocardiograms of animals over 1½ years of age and for animals with heart rates in the range of 50 to 90 beats per minute.

(4) The form and occurrence of the various deflections is discussed. Briefly, P and T were frequently of the diphasic type. P was infrequently absent. R was invariably present in leads II and III; Q occurred with great frequency and was usually quite small. S waves were not particularly numerous. The form of QRS showed considerable variability. The forms of this complex which were observed have been grouped into several different types. All these deflections showed minor variations from month to month, particularly QRS and T. A classification of the bovine electrocardiogram based on the form of QRS in lead II is suggested.

(5) The potential of the several deflections was generally small.

(6) The electrical axis of QRS ranged from $+30°$ to $+180°$ and from $-30°$ to $-170°$. Fifty percent of these were in the $+30°$ to $+90°$ range.

(7) The electrocardiograms of the various individuals were apparently unaffected by the breed of the animal. No characteristics peculiar to any one breed were observed. Similarly, the stage of gestation or of lactation exerted no apparent effect.

From the data, presented the following conclusions seem warranted:

(1) Bovine electrocardiograms can be classified into four main groups, the form of QRS in lead II being used as a basis for separation.

(2) In only one respect does the bovine electrocardiogram closely resemble that of the human subject. The interval lengths are nearly alike in the two species, which indicates that, considering the greater mass of the bovine heart, the spread of the impulse is relatively faster in this species. In all other respects no very close resemblance was observed. The form of the several deflections was in general markedly different and the potential of these deflections were smaller in the bovine than in the human subject.

(3) Variations in interval length and in the form and potential of the various deflections occur much more frequently in the bovine than in the human electrocardiogram. For this reason the significance of changes in size of potential as an indication of the nutritive state of the bovine heart is somewhat doubtful. Likewise changes in the form and direction of T waves has not the same significance that it has in the human subject.

(4) The electrical axis of QRS varied within a very wide range, and with the methods at the writers' disposal for its determination, no very definite limits could be set.

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PYTHIUM DISEASE OF FIBROUS-ROOTED BEGONIA AND ITS CONTROL[1]

By JOHN T. MIDDLETON, *junior plant pathologist, California Agricultural Experiment Station;* C. M. TUCKER, *chairman, Department of Botany, Missouri Agricultural Experiment Station;* and C. M. TOMPKINS, *assistant plant pathologist, California Agricultural Experiment Station.*

INTRODUCTION

A serious crown and stem disease of fibrous-rooted begonia (*Begonia semperflorens* Link and Otto) plants, variety Fire Sea, was observed in out-of-door plantings at Berkeley, Calif., in July 1937. During the following winter, a similar disease was found on Carmine, Christmas Cheer, and Primadonna fibrous-rooted begonia plants in a greenhouse at Columbia, Mo. A preliminary report on this disease has already been published (*4*)[2]; this paper presents more complete results of a joint investigation of this disease.

REVIEW OF LITERATURE

Only a few reports appear in the literature of a begonia disease which resembles the condition found by the writers.

In 1925, Braun (*1*) reported successful inoculation of begonia plants with cultures of *Pythium debaryanum* Hesse, *P. debaryanum* var. *pelargonii* Braun, and *P. splendens* Braun, which produced a soft, darkened stem rot and subsequent collapse of the plants. Pape (*5*) in 1927 reported a decay of petioles and blades of the Gloire de Lorraine begonia (*B. socotrana* Hook.$\times$ *B. dregei* Otto and Dietr.) in Germany and indicated that *P. debaryanum* was probably responsible for the propagation disease. More recently Pape confirmed this report in Fotsch (*3*) and added that this and other species of *Pythium* were involved. Flachs (*2*) also reports *P. debaryanum* to be responsible for a seedling disease of *Begonia* sp. in Germany. Sharples (*6*), in 1930, recorded a rot of an unidentified species of begonia, caused by *P. splendens*, occurring in Malaya. A disease of fibrous-rooted begonia occurring in the United States and comparable with the one now under discussion was described by Swift (*7*) in 1932. She found that the crown and soft stem rot of begonia was caused by a fungus identified as *P. debaryanum*.

SYMPTOMS OF THE DISEASE

The disease affects the crown, basal stem, and leaves of the plants. Symptoms of the disease are identical for the varieties Carmine, Christmas Cheer, Fire Sea, and Primadonna. In naturally infected plants, the crown and basal portion of the stem become somewhat discolored, water-soaked, and soft. Within a day or two, under

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[2] Italic numbers in parentheses refer to Literature Cited, p. 95.

favorable conditions for the development of the disease, infection may progress upwards 2 or 3 inches, causing the stem to collapse (figs. 1 and 2). The basal leaves are often infected in severely diseased specimens. The petioles become limp and dark, the blades water-soaked and flaccid; subsequent abscission of infected leaves is not uncommon. The fibrous roots, in contrast to the susceptible stems and leaves, are usually not attacked, infection of the plant generally taking place via the crown.

Young succulent begonia plants are more susceptible than old, woody plants. This may be accounted for by the fact that the fungus is confined largely to the parenchymatous cells of the cortex and stele. In older plants considerably more vascular tissue is present which impedes the progress of the pathogen.

It was noted that when potted plants were grown in close proximity to one another the disease assumed considerable importance. In such cases, the disease is distributed throughout the plant by means of leaf infection, since the lower leaves of the plants are usually in contact with each other. When similar plants were set apart the disease developed only occasionally. Excessive watering was found to be conducive to the development of the disease, especially when the plants were watered by a sprinkling system. Temperatures favorable to infection range from 60° to 70° F.

THE CAUSAL FUNGI

Isolations made from the Berkeley, Calif., material yielded a pythiaceous fungus which formed numerous acrogenous, smooth, spherical, thin-walled sporangia which measured 18 to 22 micra in diameter. The sporangia germinated by the production of one to four germ tubes. Occasional terminal, smooth, spherical, thin-walled oogonia were observed when the fungus was grown on potato-dextrose agar. These were usually indistinguishable from the sporangia and only positively identified as oogonia when oospores were contained therein. The antheridia were monoclinous, one to an oogonium, somewhat swollen, short and upcurved, the tip in narrow contact with the oogonium. The antheridia always originated from the oogonial stalk immediately below the oogonium. Oospores were smooth, spherical, thick-walled, not completely filling the oogonial cavity. The fungus was identified as *Pythium ultimum* Trow.

So far as the writers are aware, this is the first record of *Pythium ultimum* from begonia.

Isolations from the Columbia, Mo., material yielded three pythiaceous organisms. One of these was identical with the Berkeley isolate, *Pythium ultimum*. One other produced numerous acrogenous and intercalary sporangia which germinated by means of one to five germ tubes. The terminal sporangia were somewhat larger in diameter than mentioned above for *P. ultimum*, and measured 21 to 26 micra. The intercalary sporangia were barrel-shaped. The oogonia produced when the organism was cultured on potato-dextrose agar were largely terminal, though occasionally intercalary. Acrogenous oogonia were smooth, spherical, and thin-walled. The several antheridia produced were both monoclinous and diclinous. When monoclinous they did not arise immediately below the oogonium on the oogonial stalk as stated in *P. ultimum*, but from a more distal

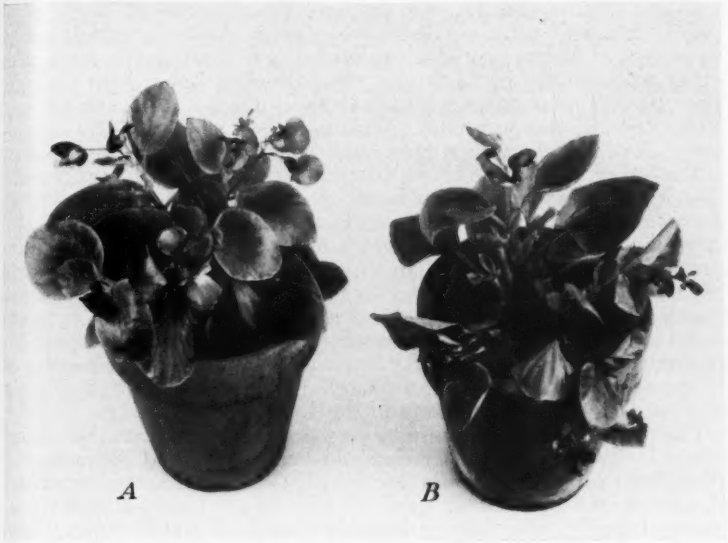

FIGURE 1.—Pythium disease of fibrous-rooted begonia: *A*, Non-inoculated control; *B*, symptoms produced on fibrous-rooted begonia plant, variety Fire Sea, by *Pythium ultimum* after inoculation in the greenhouse.

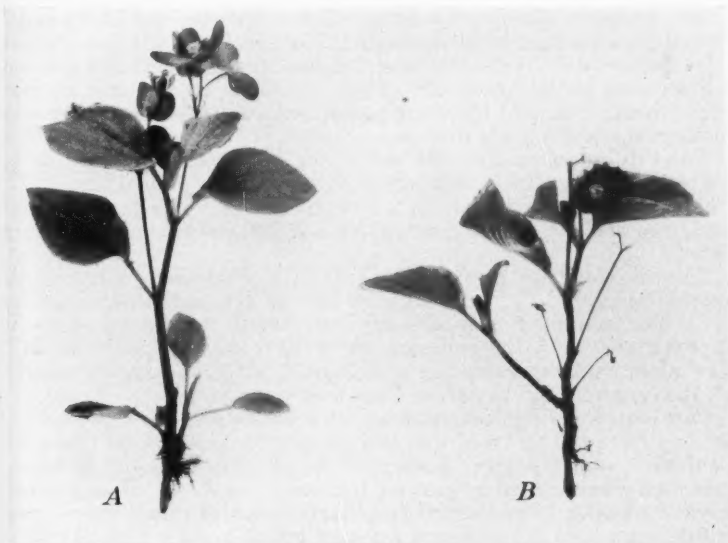

FIGURE 2.—Pythium disease of fibrous-rooted begonia: *A*, Stem from noninoculated control; *B*, symptoms produced on stem of fibrous-rooted begonia plant by *Pythium ultimum* after inoculation in the greenhouse.

position. When one to two antheridia were present, both were usually monoclinous. However, when more than two were present some were of diclinous origin. In no instance were all the antheridia either monoclinous or diclinous when three or more were present. The oospores were smooth, spherical, fairly thick-walled, and did not completely fill the oogonium. This organism conformed to the description of *P. debaryanum* Hesse and was identified as that species.

The other fungus produced an abundance of smooth acrogenous, spherical, thin-walled sporangia which measured 32 to 40 micra in diameter. The contents were dense and finely granular; one to two large oil globules were present which measured 4 to 7 micra in diameter. Germination occurred by the production of one to five germ tubes. Though sexual reproduction was not observed, the sporangia were so conspicuously different from those of other species of *Pythium* found in this group that it was identified as *P. splendens* Braun. This determination was confirmed by a comparison with known isolates of *P. splendens*. This is the first record of *P. splendens* from naturally infected fibrous-rooted begonia in the United States.

The relation of temperature to growth of the mycelium was determined for two isolates of *Pythium ultimum*, one from California, the other from Missouri, and for one isolate each of *P. debaryanum* and *P. splendens*. The culture tubes (2.1 by 20 cm.) used and the procedure followed are those previously described by Tompkins and Gardner (*8*). Each tube is provided with a glass dam at the open end; 13 ml. of Difco corn-meal agar (pH 6.0) is added to each tube and allowed to cool to a uniform depth in a horizontal position, the dam preventing the escape of the liquid agar.

The tubes are inoculated near the dam at the open end with a small potato-dextrose agar block containing the mycelium of the fungus cut from 3-day-old Petri dish cultures. Inoculated tubes are left at room temperature for 24 hours, the extent of growth at the close of that period being indicated by a wax-pencil mark on the tube; subsequent measurements are made from this point.

Three tubes of each isolate are placed in a horizontal position in controlled temperature chambers at intervals of 3° from 1° to 43° C. The cultures are incubated for a 72-hour period. The cardinal temperatures are based on the extent of mycelial growth in the culture tubes.

The minimum temperature for growth of *Pythium debaryanum* is approximately 4° C., the optimum 28° to 31°, and the maximum 37°. The minimum temperature for growth of *P. splendens* is approximately 10°, the optimum 28° to 31°, and the maximum 34°. The minimum temperature for growth of *P. ultimum* is approximately 4°, the optimum 25° to 28°, and the maximum 37°.

Pure cultures of *Pythium ultimum*, *P. debaryanum*, and *P. splendens*, isolated from the stems of diseased, fibrous-rooted begonia plants in California and Missouri, were used in the infection experiments. Inoculum was prepared by growing the isolates on sterilized, moistened cracked wheat. It was added in uniform quantity to autoclaved soil in 6-inch pots, each containing a young plant, variety Fire Sea, in a manner designed to avoid wounding the roots or stem. Sterilized cracked wheat was used for the noninoculated controls. The plants, grown in a greenhouse at temperatures ranging from 55° to 65° F., were watered heavily each day to provide favorable conditions for

infection. Of 20 plants inoculated with each species of *Pythium*, all became diseased, while the 20 control plants remained healthy. The fungus was reisolated from each infected plant, and the reisolates proved pathogenic when tested on healthy plants. Symptoms on artificially infected plants were identical with those of naturally infected plants.

EXPERIMENTAL HOST RANGE

The experimental host range was determined only for *Pythium ultimum*, and the technique involved was that described for the pathogenicity tests. Reisolations were made from all diseased plants, and the reisolate of the fungus from a particular host was then tested again by inoculation.

As it is generally conceded that *Pythium ultimum* attacks a large variety of hosts, it was thought advisable to try to ascertain its pathogenicity to a wide selection of plants, many of which might be grown in conjunction or rotation with fibrous-rooted begonia. The fungus proved pathogenic to young plants of eight species in eight genera representing eight families: Spinach (*Spinacia oleracea* L.) var. Bloomsdale, sweet-william (*Dianthus barbatus* L.), rocket larkspur (*Delphinium ajacis* L.), cauliflower (*Brassica oleracea* L. var. *botrytis* L.), mignonette (*Reseda odorata* L.), *Godetia grandiflora* Lindl., *Schizanthus pinnatus* Ruiz and Pav., and cucumber (*Cucumis sativus* L.). Perhaps the host range could have been extended if only very young seedlings had been used or if seeds had been sown in experimentally infested soil. This technique would reveal the ability of the fungus to cause damping-off and would not necessarily be indicative of its efficacy as a root- or stem-rotting agent of more mature plants.

No infection was obtained on the following plants, representing 34 species in 32 genera of 19 families:

Aizoaceae:
- Iceplant (*Mesembryanthemum crystallinum* L.)

Amaranthaceae:
- Cockscomb (*Celosia argentea* L. var. *cristata* Kuntze)

Campanulaceae:
- Canterbury-bells (*Campanula medium* L.)

Caryophyllaceae:
- Carnation (*Dianthus caryophyllus* L.)

Compositae:
- Transvaal daisy (*Gerbera jamesonii* Hook. var. *transvaalensis* Hort.)
- China-aster (*Callistephus chinensis* Nees) var. Giant Branching White, wilt resistant
- French marigold (*Tagetes patula* L.)
- African marigold (*Tagetes erecta* L.)
- Hybrid cineraria (*Senecio cruentus* DC.)
- *Gaillardia pulchella* Foug. var. *picta* Gray

Cruciferae:
- Cabbage (*Brassica oleracea* L. var. *capitata* L.)
- Radish (*Raphanus sativus* L.)
- Dames violet (*Hesperis matronalis* L.)
- Annual stock (*Matthiola incana* R. Br. var. *annua* Voss)

Dipsacaceae:
- Mourning bride or pincushion flower (*Scabiosa atropurpurea* L.)

Labiatae:
- Flowering sage (*Salvia farinacea* Benth.)

Leguminosae:
- Broadbean (*Vicia faba* L.)

Malvaceae:
- Hollyhock (*Althaea rosea* Cav.)

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Papaveraceae:
Iceland poppy (*Papaver nudicaule* L.)
Oriental poppy (*Papaver orientale* L.)
California poppy (*Eschscholtzia californica* Cham.)
Polemoniaceae:
Phlox drummondii Hook.
Ranunculaceae:
Poppy anemone (*Anemone coronaria* L.)
Love-in-a-mist (*Nigella damascena* L.)
Hybrid delphinium (*Delphinium cultorum* Voss)
Meadowrue (*Thalictrum dipterocarpum* Franch.)
Rosaceae:
Geum chiloense Balb.
Scrophulariaceae:
Snapdragon (*Antirrhinum majus* L.)
Pentstemon or beardtongue (*Pentstemon barbatus* Nutt.)
Solanaceae:
Tomato (*Lycopersicon esculentum* Mill. var. *vulgare* Bailey) var. Early Santa Clara Canner
Tobacco (*Nicotiana tabacum* L.) var. Turkish
Tropaeolaceae:
Garden nasturtium (*Tropaeolum majus* L.)
Umbelliferae:
Celery (*Apium graveolens* L. var. *dulce* DC.)
Violaceae:
Pansy (*Viola tricolor* L.)

Pure cultures of *Pythium debaryanum*, *P. splendens*, and *P. ultimum*, when added to 6-inch pots containing sterile soil and sown to tomato, caused approximately 90 percent damping-off.

DISCUSSION

In commercial practice, fibrous-rooted begonia plants are propagated from cuttings. Since the causal organisms are soil-borne fungi, the disease may be controlled in the greenhouse by steam sterilization of flats and soil. Also, proper spacing and careful watering of plants are necessary if the disease is to be controlled in the early stages of growth of rooted cuttings.

When transplanted to beds out of doors, the young begonia plants are again exposed to infection from the soil. The degree of infection could be materially lowered by keeping the plants "on the dry side." All varieties tested with *Pythium ultimum* in the greenhouse in Berkeley showed some degree of susceptibility, with the exception of a pink-flowering type which appears to be immune. Repeated attempts, under experimental conditions, to infect this variety have yielded negative results. It is immune to the disease in the open as well as in the greenhouse. It has been impossible to obtain the name of this hybrid, even with the assistance of recognized authorities on begonias, and the writers therefore propose that it be named "Calmo."

SUMMARY

A destructive crown and stem disease of fibrous-rooted begonia plants, prevalent in California and Missouri, is described.

The symptoms consist of a watery rotting of crown, stem, petiole, and leaf tissues. Infection may spread upward several inches above the soil line, eventually causing the stem to collapse. The fibrous roots are usually not attacked.

Overcrowding of plants, excessive watering, and relatively high temperatures predispose begonia plants to infection.

In California, the causal fungus is *Pythium ultimum*, while in Missouri, three species are involved, namely, *P. debaryanum*, *P. splendens*, and *P. ultimum*. These isolates are described in detail. All proved pathogenic to healthy fibrous-rooted beognia plants, variety Fire Sea.

The temperature-growth relations of the organisms were determined. The values for *Pythium debaryanum* are: Minimum 4° C., optimum 28° to 31°, maximum 37°; the values for *Pythium splendens* are: Minimum 10°, optimum 28° to 31°, maximum 34°; the values for *Pythium ultimum* are: Minimum 4°, optimum 25° to 28°, maximum 37°.

Pythium ultimum was also pathogenic to spinach, sweet-william, rocket larkspur, cauliflower, mignonette, *Godetia grandiflora*, *Schizanthus pinnatus*, and cucumber. No infection was obtained on 34 additional species of plants in 32 genera belonging to 19 families.

The disease may be controlled in the greenhouse by using flats and soil which have been steam-sterilized. For out-of-door plantings, an immune variety of fibrous-rooted begonia is available.

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METHODS OF SPLITTING HEVEA SEEDLINGS[1]

By H. F. Loomis

Associate agronomist, Rubber Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture[2]

INTRODUCTION

Doubling the number of small plants of the Pará rubber tree (*Hevea brasiliensis* Muell. Arg.) through vertical division of very young seedlings into two or three parts has been reported by several experimenters in the Netherlands Indies. One of the most recent and most thorough studies of the two accepted methods devised for splitting seedlings is that reported by Zweede.[3] He used what is known, from its inventor, as the Ramaer method of division, in which very young seedlings are split into two more or less equal parts by a vertical cut passing through the main-stem sprout and taproot between the cotyledon petioles (fig. 1). Thus each section remains attached to the seed by one of the cotyledon petioles. Zweede reported that both sections of the main stem always die as a result of equal division, but that each is replaced, after some delay, by a shoot developed from the bud in the axil between the cotyledon petiole and the main stem. When the taproot has been equally divided, these two cotyledon shoots develop evenly; unequal division of the taproot results in the survival of both sections but in the more rapid growth of the shoot that has the larger amount of taproot. Unequal division of the top results in the death of the smaller section and the survival of the larger.

The other accepted method of division, also named after its originator, is the Gambar method, in which the vertical cut does not completely divide the main stem but begins at a point slightly above the axil formed by one of the cotyledon petioles with the main stem and passes obliquely inward to the center of the main stem and downward between the cotyledon petioles, dividing the taproot into two approximately equal parts (see fig. 10). The result is two unequal sections, each attached to a cotyledon petiole. One section comprises the main stem, with a basal chip removed, and half of the taproot with its developing lateral roots; the other and smaller section comprises only the chip with a cotyledon bud, from the main stem, and the other half of the taproot with its growing lateral roots. This method of division has been found more satisfactory than the Ramaer method, as the main-stem portion grows much more rapidly than either Ramaer section, while the axillary shoot grows as fast or faster than the Ramaer sections.

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[2] The investigations herein reported were carried out while the author was a member of the staff of the Division of Plant Exploration and Introduction, Bureau of Plant Industry.

[3] Zweede, J. C. Stemmen uit de practijk het splitsen van hevea-kiemplanten. Bergcultures 14: 1271–1275, illus. 1940.

Lammers,[4] another Netherlands investigator, has reported a modification of the Gambar method, whereby triplets instead of twins were produced. He divided seedlings into three parts, two of which were formed by slicing off each of the cotyledon buds together with about one-third of the taproot; the third part comprised the central section or third of the taproot, and the main stem from which the cotyledon bud on each side had been removed. This division left each cotyledon bud chip and its portion of taproot attached to the seed but left the main-stem section completely detached from the seed and the necessary food supply. The cotyledon-bud sections were much the same as those produced by the Gambar method except that they had somewhat less taproot, one-third instead of one-half.

Lammers considered the production of triplets impracticable for commercial purposes because of the relatively high initial loss of seedlings, resulting from inaccuracies of splitting, and the high mortality of the sections later, particularly those of the main stem, which, unlike axillary sprouts, receive no nutrition from the seed. The final result of his triplet method was fewer plants than would have been secured by either the Ramaer or the Gambar method.

EXPERIMENTAL PROCEDURE

In the spring of 1941 a small number of fresh hevea seeds became available for experimental work at the United States Plant Introduction Garden, Coconut Grove, Fla. With these seeds both the Ramaer and the Gambar methods of division were studied, as well as several modifications of them which apparently had not been used before. In spite of the relatively few seeds which it was possible to allot to each test, the results and behavior in many cases appear sufficiently interesting to justify report.

In all tests, seedlings for division or control purposes were of approximately the same age, the plumule having been withdrawn from the seed not more than 24 hours previously. Examples of seedlings at this stage are shown in figures 1, 10, and 15. The split seedlings and the controls were planted in a mixture of sand and peat moss in 4-inch pots and were placed in partial shade in a glasshouse, where they were watered once a day. The split seedlings remained in these pots until the first flush of leaves had developed and had assumed the nearly horizontal position of mature leaves. The twin plants were then separated and placed in individual 4-inch pots, where they remained for a week or 10 days before being set in the field nursery. Throughout the work any of the split seedlings that reached the stage where they were separated and repotted were considered as established plants for record purposes, insofar as the success of the dividing operation was concerned.

RAMAER METHOD

TESTS

Forty newly germinated seedlings of *Hevea brasiliensis* were carefully divided by the Ramaer method, as shown in figure 1, special care being exercised to divide as equally as possible the tip of the main stem and the tip of the taproot. Of these 80 sections, 78 survived

[4] LAMMERS, IR. R. P. EENIGE VERDERE GEGEVENS OVER HET SPLITSEN VAN HEVEA KIEMPLANTEN. Bergcultures 15: 397–398, illus. 1941.

FIGURE 1.—Hevea seedling with plumule withdrawn from the seed within the previous 24 hours. Splitting of the entire seedling, with special care to divide equally the tip of the plumule and of the taproot, is typical of the Ramaer method of dividing seedlings. × 3½.

as separate plants, having been produced either as a continuation of the main stem or, when the main stem died, as a sprout from the bud in the axil between the cotyledon petiole and the main stem. In only 2 cases did split seedlings fail to produce 2 plants. In each case the main stem had died and it was found that no axillary bud was present, not merely that it had failed to sprout. The surviving half of 1 of these 2 seedlings developed from the split main stem; the survivor of the other seedling developed from the cotyledon bud. Twin plants were derived from the other 38 seedlings as follows:

Three split seedlings produced a plant from each main-stem section, thus yielding six plants.

Each of 7 seedlings produced 1 plant from the main-stem section and another plant from the opposite cotyledon bud, the main stem on that side having died; thus 14 plants were produced.

On 28 seedlings all main-stem sections died, but a plant sprang from the cotyledon bud of each section, thus producing 56 plants.

It was observed that plants produced from the main-stem sections, whether from both sections of a seedling or from only one, usually were badly bent over at first (fig. 2) and grew very slowly, being soon

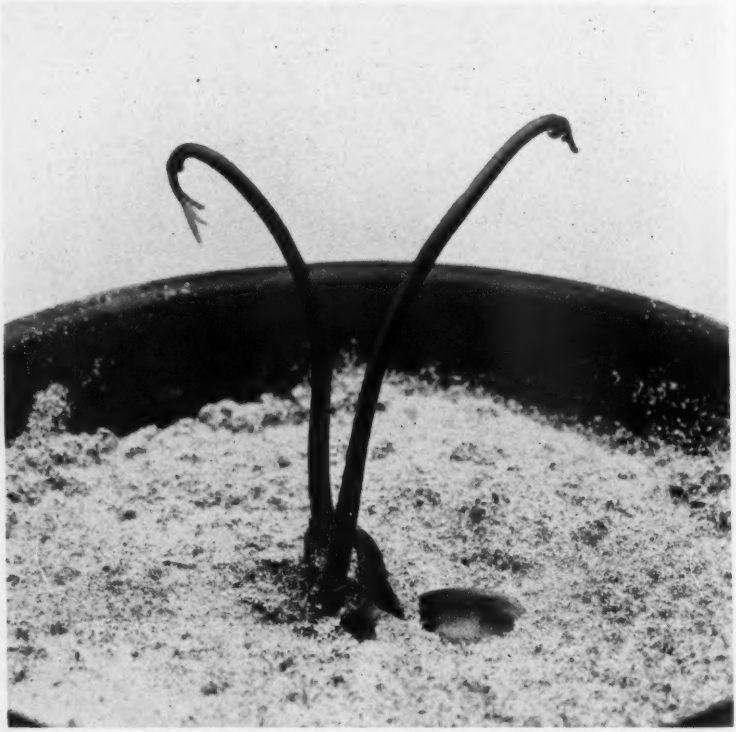

FIGURE 2.—The two main-stem sections of seedling 19, split by the Ramaer method 11 days previously, showing the typical bent condition of growing main stems sectioned by this method. × 1 approximately.

outdistanced by cotyledon shoots of the same age (fig. 3), and that the first leaflets were reduced in number and malformed (fig. 4). Several cases were observed in which the main-stem section lived but

FIGURE 3.—Cotyledon shoot (left) replacing dead main-stem section, and the surviving main-stem section (right) of a seedling divided by the Ramaer method 35 days previously. The stunting of main-stem growth is typical of this method, while normal growth of the cotyledon shoot enables it to outdistance the main-stem shoot in a relatively short time. × ½.

FIGURE 4.—Two seedlings, *A* and *B*, divided by the Ramaer method 35 days previously, with both their main-stem sections growing. Retarded growth, reduction and malformation of leaflets, typical of main-stem sections produced by this method, are evident. *B* is seedling 19, shown at an earlier stage in figure 2. × ⅔ approximately.

failed to send out leaves at the tip as is usual, later produced a new flush of growth surmounted by normal leaves (figs. 5 and 6).

FIGURE 5.—Growing main-stem sections of a seedling 42 days after it was divided by the Ramaer method. The section on the right has sent up, from its tip, a single leaf of two parts instead of three as is normal, while the section at the left did not send up any leaves at its tip but did produce a new flush of growth surmounted by two immature but entirely normal compound leaves. × 1 approximately.

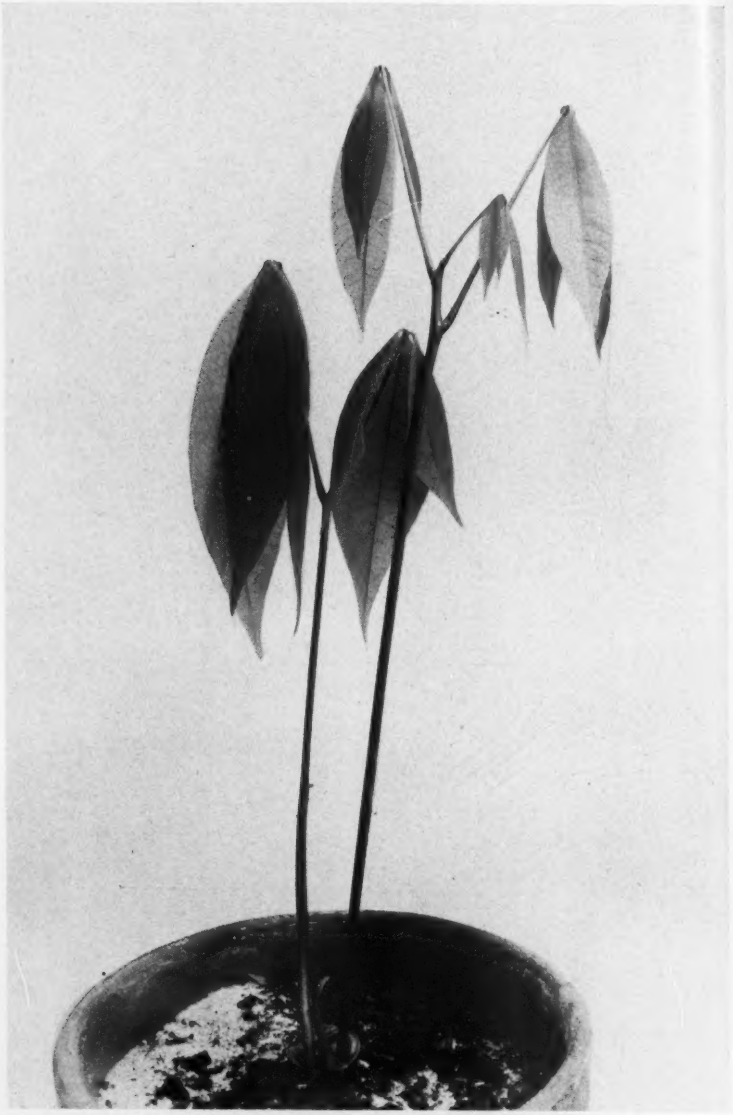

FIGURE 6.—Seedling divided by the Ramaer method 28 days previously. Main-stem section (right) alive but dormant, its cotyledon bud being forced into growth. Surviving main-stem section (left) shows slower growth than the cotyledon shoot and is unusual in that it has normal leaves. ×<1.

In instances where the main stem was slow in dying, sometimes remaining alive for a month or 6 weeks, the bud in the axil between main stem and cotyledon petiole appeared to sprout more slowly than where the main stem died promptly, and the resulting shoot appeared to grow more slowly than where main-stem tissue was not being supported by food from the cotyledon (fig. 7). There were not enough instances of this, however, to allow application of statistical methods for determining this point.

At the time when the 78 plants produced by the Ramaer method were placed in separate pots, they averaged 41.7 days from date of splitting. Those which had grown from main-stem sections averaged 84.6 mm. in height with taproots 122.0 mm. long, whereas plants from cotyledon buds averaged 121.1 mm. in height with taproots 118.7 mm. long.

It is interesting to note that Zweede,[5] reporting on approximately 5,000 seedlings divided by the Ramaer method, stated that when the plumule was exactly halved both main-stem sections always died and usually were replaced by cotyledon shoots, but that when main-stem division was unequal, as one-quarter and three-quarters, the larger section lived but the smaller one died. In the tests conducted at Coconut Grove, the plumule or main stem was approximately halved in all 40 seedlings; 3 of these seedlings produced twin plants from main-stem sections, while in 7 other seedlings 1 of the twin plants derived from each was from the main-stem section.

MODIFICATION

In an attempt to eliminate as much as possible the effect of the dormant main-stem section on its basal cotyledon bud, 20 seedlings were split by the Ramaer method, but at the same time the main stem was cut off just above the cotyledon bud. This treatment resulted in producing 39 plants that did not seem to be greatly different in behavior from cotyledon plants produced by the unmodified Ramaer method except that the twin plants usually were more uniform in growth (figs. 8 and 9). When separated and repotted the plants averaged 116.5 mm. in height with taproots 97.7 mm. long. From data given on page 110, it will be seen that these plants equaled Gambar cotyledon sections but not Gambar main-stem sections. A comparative test of a larger number of seedlings divided by the Ramaer method, part of which should have the main stems cut off at the time of division, probably would show that the development of the latter group was the more rapid.

GAMBAR METHOD

TESTS

Twenty seedlings split by the Gambar method (fig. 10) yielded 36 plants from the 40 sections. Of the 4 sections that failed to develop, 2 were cotyledon sections in which no axillary bud appeared to be present; the other failures were the 2 parts of a divided seedling that died before the leaves developed.

[5] See footnote 2.

FIGURE 7.—Seedling divided by the Ramaer method 26 days previously. Main-stem section on left dead and replaced by a shoot from the cotyledon bud. Main-stem section on right is alive but has remained dormant, forcing a sprout from the cotyledon bud which is only half as tall as the opposite cotyledon sprout. $\times > 1$.

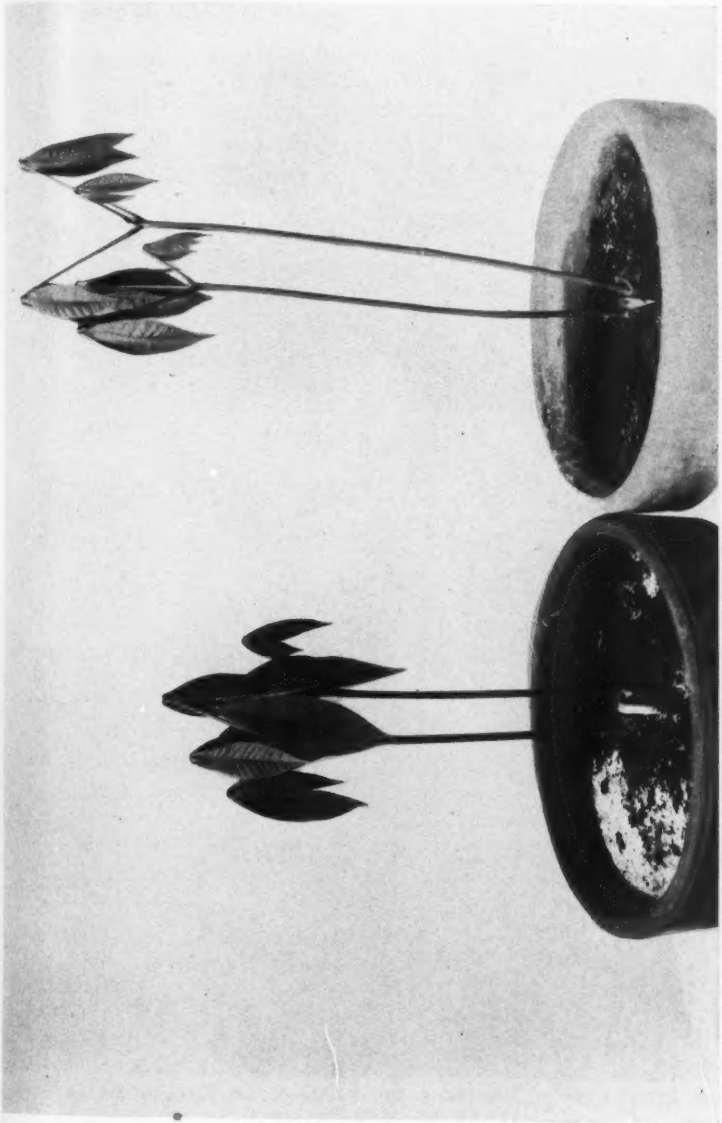

FIGURE 8.—Two seedlings divided by the Ramaer method 26 days previously, when the main stems were cut off just above the cotyledon buds to force them into growth. The uniform development of the cotyledon shoots is characteristic of this treatment. × ⅓.

FIGURE 9.—The two uniform cotyledon shoots of a seedling divided by the Ramaer method 40 days previously, when the main-stem sections were cut off to force the cotyledon buds into growth. × ½ approximately.

FIGURE 10.—Gambar method of splitting a seedling: Hevea seedling with plumule withdrawn from the attached seed within the previous 24 hours. × 4.

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Twin plants produced by the Gambar method were ready for separation at an average of 27.2 days after the seedlings were split, whereas Ramaer twins were separated at an average of 41.7 days. The average height of the main stem of Gambar sections when the

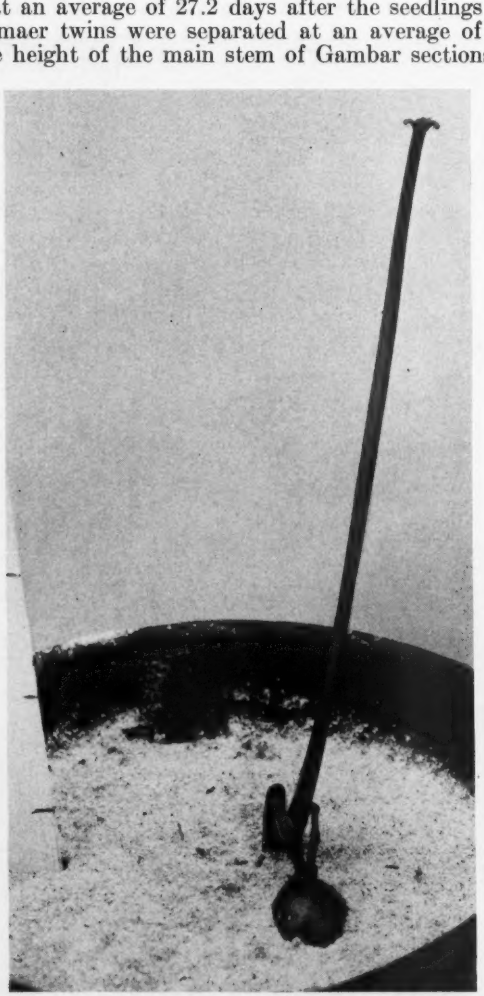

FIGURE 11.—Seedling 11 days after it was divided by the Gambar method; cotyledon bud just beginning to grow on the basal section at left. × ¾.

twin plants were separated averaged 179.4 mm., the taproots being 102.5 mm. long; the height of the cotyledon sections averaged 96.0 mm., the taproots also being 96.0 mm. long. Successive stages in the growth of twin plants produced by the Gambar method are shown in figures 11 to 14; in figure 14 twin plants are shown just after they had been cut apart to be potted separately. The equal development of the taproots and lateral roots is characteristic of plants produced

by either the Ramaer or the Gambar method, where the taproot was approximately halved when the seedling was split.

In both the Ramaer and Gambar methods the two sections of the split seedling are, for a time after splitting, dependent on the cotyledons for food. So far as the writer is aware, little information is available that shows how soon after the splitting of hevea seedlings the twin plants may be separated by cutting one of them, usually the larger of the two sections, loose from its cotyledon without endangering its life.

FIGURE 12.—Closer view of the basal section of the divided seedling shown in figure 11. The swelling of the cotyledon bud in the axil of this section is evident. × 3½.

In order to gain some knowledge on this point, the main-stem section of each of a number of seedlings, divided by the Gambar method, was cut loose from the cotyledon at different intervals after the seedlings were split. Fifteen split seedlings had their main-stem sections severed from the cotyledon immediately after the seedling was split and planted. Other small groups of seedlings had their

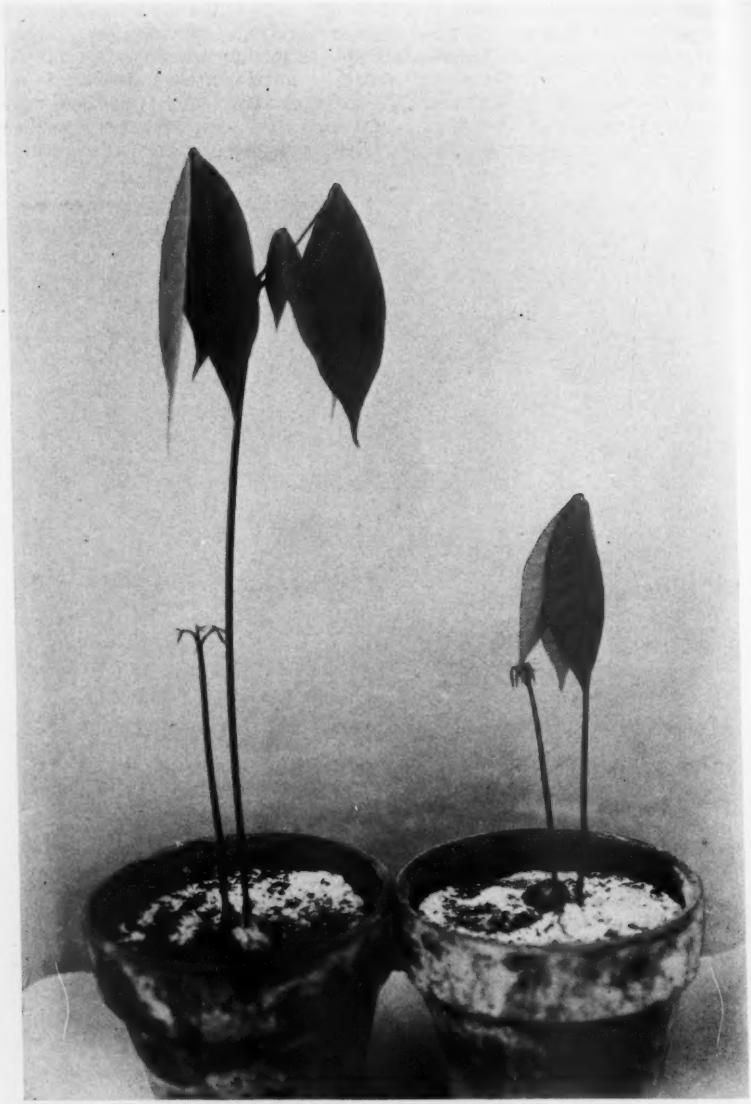

FIGURE 13.—Two seedlings divided by the Gambar method, one 22 days previously (left) and the other 23 days previously (right). The main-stem sections have ceased growth and are maturing their leaves, while the cotyledon shoots, with very young leaves, still are actively elongating. × <½.

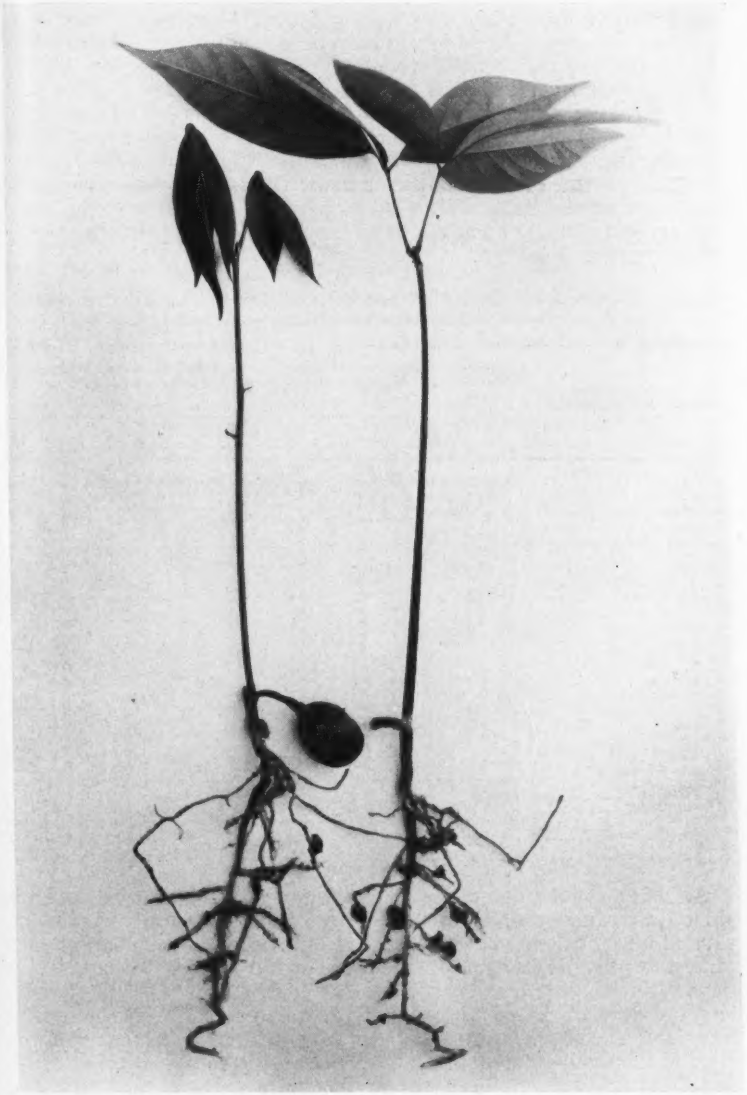

FIGURE 14.—The two parts of a seedling divided by the Gambar method 35 days previously. The two sections have been removed from the pot, cut apart, and now are ready for separate potting. The nearly exhausted seed was allowed to remain attached to the cotyledon shoot, which has attained the same height as the main-stem section but has not had time to mature its leaves. This seedling is shown at an earlier stage in figures 11 and 12. $\times <½$.

main-stem sections severed from the cotyledons at intervals ranging from 1 to 19 days after they were split. The number of main-stem sections thus treated, the period between severance from the cotyledon and death, and the number of sections of each interval group that died and the number that survived are given in table 1.

None of the plants in the first four interval groups shown in table 1 had leaves formed at the tip of the main stem. In the 9- and 10-day groups, the main stems had very small, dark purplish-brown, pendent leaves. In the 14- and 15-day groups, the leaves were much larger and were at the intermediate stage between vertical and the nearly horizontal position of normal mature seedling leaves, to which class the 19-day group belonged.

TABLE 1.—*Periods of survival of main-stem sections severed from cotyledons at various intervals after seedlings were split*

Days between severance from cotyledon and death of section	Main-stem sections surviving for various periods when the number of days between splitting of seedlings and severance of main stem from cotyledons was—								
	0	1	2	4	9	10	14	15	19
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
1	0	0	1	0	0	0	0	0	0
2	0	1	0	0	0	0	0	0	1
5	0	1	0	0	0	0	0	1	0
7	3	0	0	0	0	0	0	0	0
9	2	0	1	0	0	0	0	0	0
10	0	1	0	0	0	0	0	0	0
11	1	1	0	0	0	0	0	0	0
12	1	0	0	0	0	0	0	0	0
13	3	2	0	0	1	1	0	0	0
14	2	0	0	1	0	0	0	0	0
15	1	0	0	0	0	0	0	0	0
17	0	2	0	2	0	0	0	0	0
19	2	0	1	1	0	0	0	0	0
20	0	1	0	0	0	0	0	0	0
21	0	0	5	3	0	0	0	0	0
22	0	0	0	0	1	0	0	0	0
23	0	1	1	0	0	0	0	0	0
24	0	0	0	1	0	0	0	0	0
31	0	0	0	1	0	0	0	0	0
33	0	0	0	0	1	0	0	0	0
Deaths	15	10	9	9	3	1	0	1	1
Survivals	0	0	0	1	4	2	2	7	9
Seedlings split	15	10	9	10	7	3	2	8	10

Although the number of seedlings available for this test, as shown in table 1, was much too small for the results to be statistically significant, several observations are justified. It appears that the main stem is almost wholly dependent on the attached cotyledon for at least 4 days after seedling plants have been split, and not until they are 10 or more days old is a high percentage likely to survive if the flow of food from the cotyledon is stopped. Also it is obvious that delay in cutting the main stem loose not only decreases the likelihood of death but delays it when it does occur. In those sections that remained alive for a considerable length of time, the main stem usually ceased to elongate and remained dormant from 7 to 18 days before death occurred.

The 15 main stems cut loose from their cotyledons when the seedlings were split continued to elongate for an average of 4.7 days before the period of dormancy that preceded death, and in this time they gained an average of 3.3 mm. in height. From the data in table 1 it

may be determined that the average age of these plants from the time of severance until death was 12.1 days; hence, the average period of dormancy preceding death was 7.4 days.

In the group cut loose from the cotyledons 4 days after the seedlings were split, the nine main stems that died had continued to grow for 6.3 days before dormancy set in, and averaged 7.8 mm. increase in height. The average period between day of severance from the cotyledon and death was 20.6 days, thus making the period of dormancy before death 14.3 days. In none of the other categories in table 1 were sufficient data obtained to allow such inferences to be made.

Twenty normal seedlings that had not been split were cut loose from the cotyledons immediately after being planted. Although all of these seedlings died, they survived longer than the Gambar sections, 16 days elapsing between the time the cotyledon petioles were cut and the first three deaths, and 51 days elapsing before the last death, as shown in table 2.

TABLE 2.—*Data on 20 entire seedlings, with plumule just withdrawn from the seed, which were cut loose from their cotyledons and planted*

Seedling No.	Height of main stem at time of severance from cotyledons	Maximum height attained by main stem	Net gain in height of main stem	Period between severance and maximum height	Period between severance and death
	Mm.	*Mm.*	*Mm.*	*Days*	*Days*
141	21	30	9	9	21
142	39	65	26	12	40
143	22	30	8	12	19
144	26	35	9	12	21
145	28	42	14	11	18
146	23	30	7	8	20
147	29	38	9	16	20
148	56	60	4	8	29
149	42	60	18	16	51
150	22	32	10	8	20
151	34	40	6	11	25
152	23	38	15	14	19
153	26	35	9	7	17
154	29	40	11	11	16
155	22	44	22	16	25
156	29	65	36	16	25
157	17	30	13	6	20
158	26	35	9	6	16
159	21	30	9	6	23
160	23	42	19	6	16
Total	558	821	263	211	461
Average	27.9	41.0	13.1	10.5	23.5

Table 2 shows also that the seedlings continued to grow for an average age of 10.5 days and increased in height 13.1 mm. on an average, in both cases considerably exceeding Gambar main-stem sections cut loose on the day the seedlings were split or even those cut loose 4 days later. However, it must be remembered that while the Gambar sections retained most of the main stem, this was supported by only half the taproot and its portion of lateral roots. Moreover, all cut sections probably suffer a certain amount of shock and in healing their wounds must put forth an appreciable amount of energy that otherwise might be used in their struggle for existence. The 20 unsplit seedlings lived on an average 23.5 days after separation from the cotyledons and were dormant for a period averaging 13 days before death.

Unfortunately no additional seeds were available to permit determination of the age at which unsplit seedlings may survive on their own roots after being cut loose from the cotyledons.

In view of the fact that in the present experiments severance of the cotyledons from entire whole seedlings and Gambar main-stem sections up to several days old resulted in the death of all, it is of interest to observe that Lammers [6] reported that an indefinite number of the main-stem sections made by his triplet method survived the treatment. From his somewhat indefinite data it appears that either 4 percent or 27 percent of the total main-stem sections survived, depending on the interpretation one puts on the figures that he gave. Survival of even 4 percent of his main-stem sections is surprising in view of his method of splitting seedlings, which left the main-stem sections completely detached from the cotyledons and with even less taproot and lateral roots than in the simple Gambar method, while the double cut increased the mutilation of the sections.

MODIFICATION

A rather serious objection to both the Ramaer and the Gambar method of increasing the number of plants from a given lot of seedlings is the labor involved in separating and replanting the twin plants as soon as they are able to rely fully on their own roots for support. Also, in spite of the utmost care, some damage always is done to the small plants during this operation, and it is followed by a usually small but varying percentage of deaths depending on somewhat controllable factors, such as the stage at which separation and replanting are done, the injuries sustained at this time, and the post-planting care the plants receive in their new location.

In an attempt to see whether the two Gambar sections could be separated when the seedlings were split, and then be planted separately without further disturbance, 25 seedlings were split by this method, and at the same time the cotyledons were removed from the seed coat and split apart, each cotyledon remaining attached to the corresponding section of the seedling (fig. 15).

In removing the cotyledons, the seed was placed in a vise, and pressure was slowly applied until the hard seed coat cracked without damaging the cotyledons. The seed then was taken from the vise and the seed coat carefully removed, piece by piece, with a knife. After the cotyledons had been removed from the seed it was possible to insert a knife between them, at the end opposite the seedling, and force them apart, as they are held together only by a surrounding, spongy, tough skin, or tegmen. The seedling was then cut in two, the sections thus becoming entirely separated, and each section was planted with its attached cotyledon resting on the surface of the soil. For uniform experimental treatment, the two parts of a seedling were planted well apart in a pot having a central partition of oiled paper, to allow division of the ball of soil into two parts without disturbance to the roots of either section when repotting or planting out should become necessary, although actually, at the time of repotting, the balls of soil were broken to allow examination and measurement of the roots that had developed on each plantlet. The two well-separated parts of a split seedling, with their cotyledons on the surface of the soil, are shown in figure 16. Figure 17 shows two seedlings 47 days after similar treatment.

[6] See footnote 3.

FIGURE 15.—Hevea seedling, less than 24 hours after the plumule was withdrawn from the seed; cotyledons removed from the seed and split apart; seedling divided by the Gambar method for separate planting. × 4.

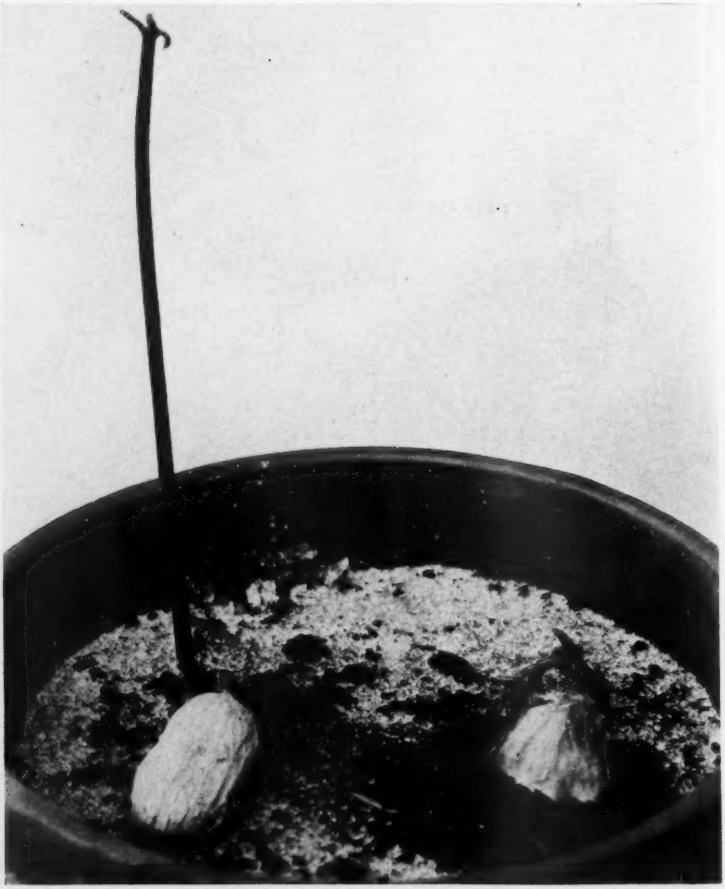

FIGURE 16.—A seedling 8 days after being divided by the Gambar method and after having the cotyledons removed from the seed and split apart and the main-stem and cotyledon-bud sections planted separately. The cotyledon bud is just beginning to send up a sprout from the small section at the right. × 1.

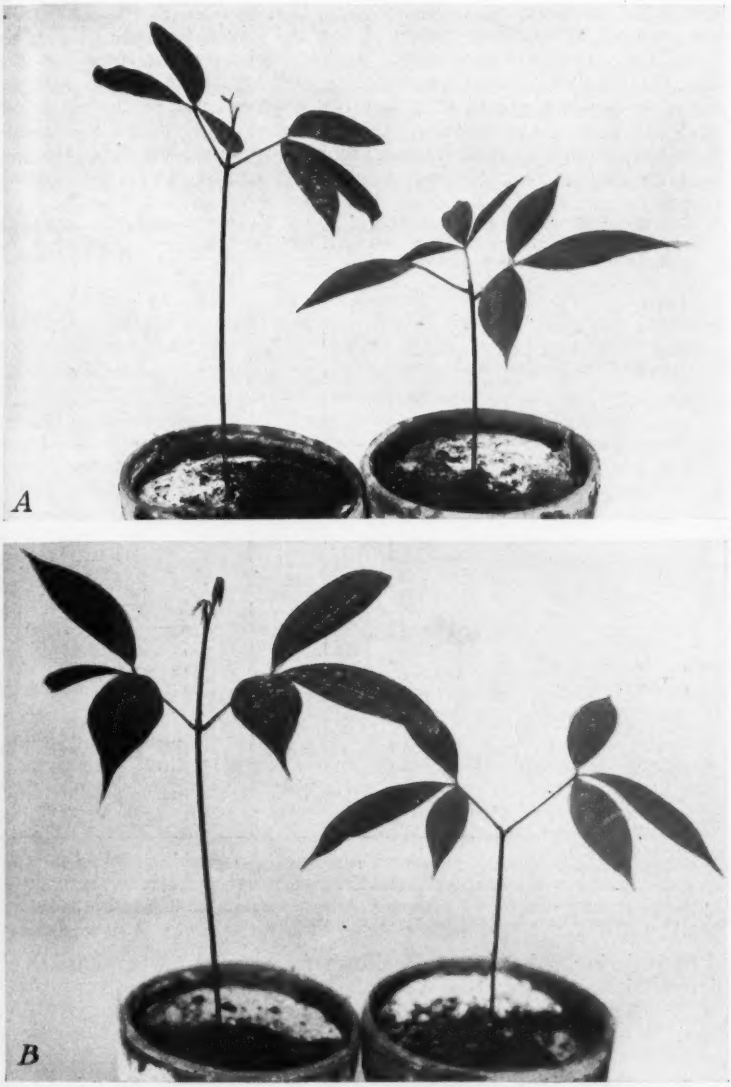

FIGURE 17.—Two seedlings, *A* and *B*, 47 days after they were divided by the Gambar method and separated by having the cotyledons removed from the seed and split apart. In each set of twins the main-stem section (left) has matured its first leaves and has started a second flush of growth not yet begun by the cotyledon sections (right), although they have matured their first leaves. ×⅓ approximately.

Division of these seedlings yielded 50 sections, 40 of which survived as plants, as shown in table 3, where it may also be seen that both sections of 16 seedlings survived, as did single sections of 8 other seedlings. In the case of only 1 seedling did both of the sections die. The behavior of these plants was little different from that of ordinary Gambar sections, except that their development was somewhat slower and the percentage of survival was lower. This latter difference, however, would be partly lessened by including in the data for Gambar sections the deaths occurring after their separation and repotting.

TABLE 3.—*Data on 25 seedlings divided by the Gambar method after having the cotyledons removed from the seed and split apart so that the two sections of each seedling might be planted separately*

Seedling No.	Date of dividing seedling	Height of main stem when divided	Length of taproot when divided	Date of separating and repotting twin plants	Height of main-stem section when repotted [1]	Length of taproot of main-stem section when repotted	Height of cotyledon section when repotted [1]	Length of taproot of cotyledon section when repotted
		Mm.	*Mm.*		*Mm.*	*Mm.*	*Mm.*	*Mm.*
161	Apr. 17	26	14		100		D	
162	do	34	10	May 26	170	70	85	110
163	do	25	10	do	110	80	105	100
164	do	14	11		D		10	
165	Apr. 18	34	8	May 26	170	110	105	110
166	do	24	10	do	180	130	95	120
167	do	23	7	do	175	70	150	130
168	do	28	8	do	150	115	20	105
169	do	25	6	do	145	100	80	100
170	do	20	7	do	100	95	35	105
171	do	21	17		D		12	
172	Apr. 19	18	8	May 26	185	115	120	75
173	do	39	8	do	210	100	130	75
174	do	29	10		D		32	
175	do	28	9	May 26	205	125	140	145
176	do	28	12	do	150	100	50	95
177	do	32	7		150		D	
178	do	27	11	May 26	155	115	60	150
179	do	24	22		D		11	
180	do	33	10	May 26	170	75	95	85
231	Apr. 24	22	8		D		125	
233	do	18	21		D		D	
235	do	21	18		125		D	
237	do	48	28	June 3	200	110	80	115
239	do	19	8	do	180	125	120	120
Average for 16 twin plants		28.3	10.0		165.9	102.2	91.9	108.7

[1] "D" indicates that the section died before the repotting stage was reached; the taproot of the surviving section was not examined or measured but was left undisturbed.

Considering only the 16 seedlings from which twin plants were derived, it will be seen that when the twins were separated, an average of 38 days after splitting, the main-stem sections averaged 165.9 mm. in height with taproots 102.2 mm. long, while the cotyledon sections were 91.9 mm. in height with taproots 108.7 mm. long. In view of the small number of seedlings in these tests, the size of the above-mentioned sections does not appear to be sufficiently different from that of typical Gambar sections to be attributed to the treatment, although it is obvious that the Gambar sections were much more precocious, being ready for repotting 27.2 days after they were split.

The behavior of the cotyledons removed from the seed coat was somewhat surprising in that none showed any rot until after the

shriveling which took place many days subsequent to their removal from the seed, when it was assumed that the shriveling was a natural consequence of withdrawal of food by the seedling. That the cotyledon itself also probably derives some benefit from the growing sprout was indicated by the fact that, when removed from the seed coat and split apart, the inner face of each cotyledon, that part not covered by the tegmen, was creamy white and remained so for several days, when it started to change to greenish yellow near the cotyledon petiole. This change continued gradually until the whole inner face of the cotyledon was dark green, the deeper color always spreading from the petiole, probably indicating that the chlorophyll was being supplied by the growing shoot.

The first shriveling of any cotyledon in the lot was noted 19 days after planting, on the main-stem section of seedling 171, which had attained a height of only 90 mm. Its cotyledon was light greenish yellow at a time when most of the other cotyledons were dark green. The cotyledon of the other section of seedling 171 also was light-colored, and the sprout was only 10 mm. high. The main stem died on May 12, when the cotyledon was seen to be rotting. On the same day the cotyledon of the other section was still unshriveled but had broken loose from the sprout, which survived without it. The possibility is suggested that injury to the sections of seedling 171 when it was split may have affected the exchange of materials between shoots and cotyledons. With other sections, shriveling of the cotyledons did not occur until later, some still being green and turgid when the sections were repotted.

SPLITTING OF OLDER SEEDLINGS

In the spring of 1934 a method was investigated of dividing hevea seedlings at a greater age than any thus far reported by other workers.[7]

The observation that nursery seedlings cut off near the ground by rats or rabbits or killed back by cold frequently survived by the sprouting of one or both cotyledon buds led to the idea that such plants, with two sprouts, might be split and made to produce two separate plants. Accordingly, seedlings ranging from 10 days to 15 months old (above ground) were cut off several inches or less above ground to force the cotyledon buds to start. Seedlings from 3 to 15 months old were split apart between the cotyledon scars immediately after topping and their parts separately planted; the younger seedlings were allowed to remain in the nursery bed until examination, 25 days later, showed that only 16.7 to 33.3 percent of the plants had produced sprouts from both cotyledon buds. These younger plants, with double sprouts, were then bisected and the sections planted separately. Figure 18 shows seedlings 35 to 50 days old ready for division, with cotyledon sprouts forced by cutting back the main stem. While both halves of a few of the younger seedlings that were divided survived the treatment, the number of plants derived did not equal the number of seedlings used in the tests; and with seedlings over 3 months or more old, scarcely any of the sections survived, one difficulty apparently being the inability or failure of the split surfaces to form calluses and prevent rotting.

[7] This method was devised and tested by the writer and Alfred Keys, formerly associate horticulturist of the Bureau of Plant Industry, stationed at this Garden.

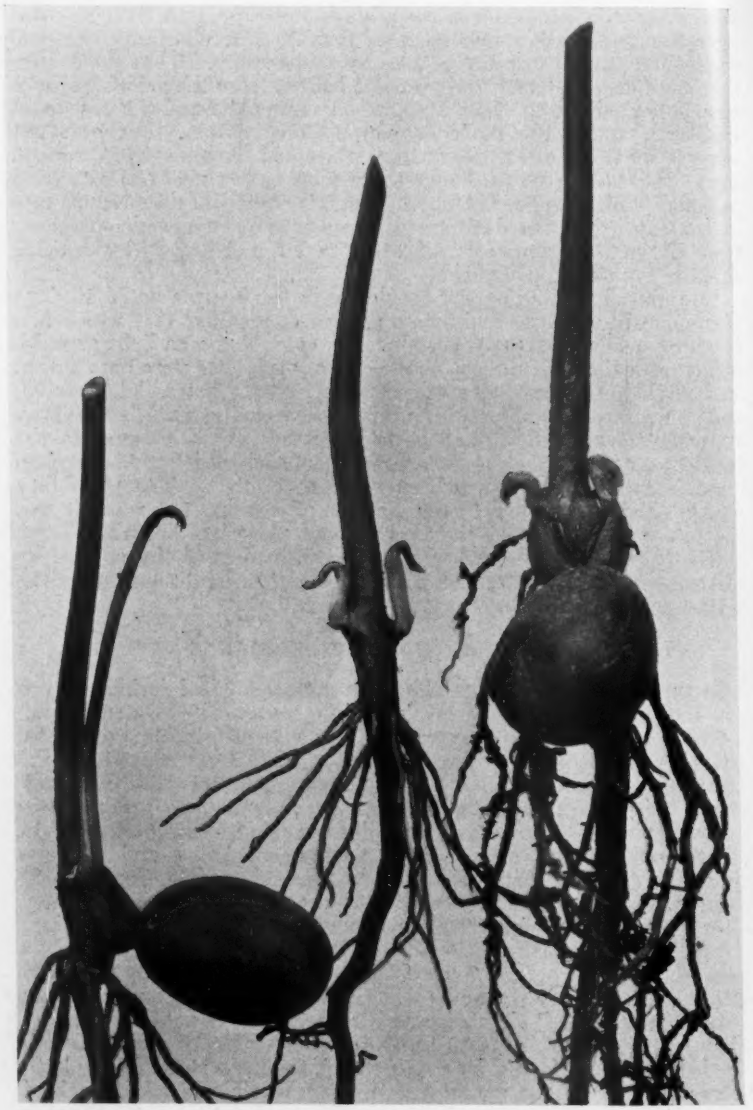

FIGURE 18.—Seedlings 35 to 50 days old from which the tops were cut 25 days previously to force the cotyledon buds seen sprouting at the base of the main stem. At this stage the seedlings were split vertically through the main-stem stump and taproot, the split passing between the cotyledon sprouts and forming two subequal sections, some of which grew, forming twin plants. × 1.

Three seedlings 3 months old, from which the tops had been cut by animals and which had produced shoots from 15 to 26 cm. high from the six cotyledon buds, were dug from the nursery, split in two, and the sections planted separately. At the time of planting the very youngest pendent leaves were removed from the tip of each shoot, leaving the growing tip and three or four old leaves intact. All six of these sections survived, which may indicate that division of older seedlings is possible if their cotyledon shoots first are allowed to develop at least one flush of leaves. One set of these divided seedlings is shown in figure 19, a year after the seedling was divided.

FIGURE 19.—Twin plants derived from splitting, a year previously, a 3-month-old seedling having a 15-cm. and a 17-cm. cotyledon sprout, each with mature leaves. These sprouts had been forced by the accidental cutting off of the seedling main stem.

SUMMARY AND CONCLUSIONS

In tests of the Ramaer and Gambar methods of dividing hevea seedlings, the conclusion of other investigators that the latter method gives better results was verified. With the Gambar method the seedlings are more easily split and the number of plants obtained is approximately as great as with the Ramaer method, but the plants are better and the time required is shorter.

Splitting of seedlings from which the main stem has been cut off just above the cotyledon bud usually results in the production of twin cotyledon plants that are the equal of plants produced from cotyledon sprouts of Gambar sections but not of those produced from main-stem sections.

Twin plants produced by the Gambar method are almost completely dependent on the food from the cotyledons for at least 4 days after they are split, and not until they are approximately 2 weeks old can a large percentage survive on their own roots when the cotyledons are removed.

In order to permit direct planting of split seedlings without the necessity of digging them up for separation and transplanting, as has to be done with Gambar and Ramaer twins, young seedlings first had their cotyledons removed from the seeds and pried apart, after which the splitting of the stem and taproot, as in the Gambar method, produced two sections which could be planted separately, each with its attached cotyledon. This method was found to be successful but not quite so productive of plants as the standard Gambar method, although the advantage of being able to plant separately the two parts of a seedling at the time of splitting may, in certain cases, offset other disadvantages.

Division of seedlings from 10 days to 15 months old was tried with fair success for the younger plants, and some indications point to the possibility of increasing the number of plants by splitting seedlings 3 or 4 months old.

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